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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 39/12, 39/00, C07K 17/00, 3/00, 13/00, 15/00, C07H 15/12, 13/00

A1

(11) International Publication Number: WO 94/22477

(43) International Publication Date: 13 October 1994 (13.10.94)

(21) International Application Number: PCT/US94/03282

(22) International Filing Date: 25 March 1994 (25.03.94)

(30) Priority Data:
08/037,816 26 March 1993 (26.03.93) US

(60) Parent Application or Grant
(63) Related by Continuation
US 08/037,816 (CIP)
Filed on 26 March 1993 (26.03.93)

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(81) Designated States: AU, BR, CA, CN, JP, RU, UA, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: HIV-1 VACCINES, ANTIBODY COMPOSITIONS RELATED THERETO, AND THERAPEUTIC AND PROPHYLACTIC USES THEREOF

(57) Abstract

The invention provides a recombinant nucleic acid molecule which encodes a mutant HIV-1 gp120 envelope glycoprotein, vaccines comprising the mutant HIV-1 envelope glycoprotein, antibodies and methods of treating individuals.

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Serial No.: 09/460,216
Filed: December 13, 1999
Exhibit 178

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HIV-1 VACCINES, ANTIBODY COMPOSITIONS RELATED THERETO, AND THERAPEUTIC AND PROPHYLACTIC USES THEREOF

Background of the Invention

Throughout this application, various publications are referenced by Arabic numerals. Full citations for these references may be found at the end of the specification immediately preceding the claims. The disclosure of these publications is hereby incorporated by reference into this application to describe more fully the art to which this invention pertains.

The life cycle of animal viruses is characterized by a series of events that are required for the productive infection of the host cell. The initial step in the replicative cycle is the attachment of the virus to the cell surface, which attachment is mediated by the specific interaction of the viral attachment protein (VAP) to receptors on the surface of the target cell. The differential pattern of expression of these receptors is largely responsible for the host range and tropic properties of viruses. In addition, an effective immune response against many viruses is mediated through neutralizing antibodies directed against the VAP. The interaction of the VAP with cellular receptors and the immune system therefore plays a critical role in infection and pathogenesis of viral disease.

The human immunodeficiency virus type 1 (HIV-1) infects primarily helper T lymphocytes, dendritic cells, and monocytes/macrophages--cells that express surface CD4--leading to a gradual loss of immune function. This loss of function results in the development of the human acquired

immunodeficiency syndrome (AIDS) (1). The initial phase of the HIV-1 replicative cycle involves the high-affinity interaction between the HIV-1 exterior envelope glycoprotein gp120 and cell surface CD4 (K_d approximately 4 x 10^{-9} M) (2). Several lines of evidence demonstrate the requirement of this interaction for viral infectivity. The introduction into $CD4^-$ human cells of cDNA encoding CD4 is sufficient to render otherwise resistant cells susceptible to HIV-1 infection (3). In vivo, viral infection appears to be restricted to cells expressing CD4, indicating that the cellular tropism of HIV-1 is largely determined by the pattern of cellular expression of CD4. Following the binding of HIV-1 gp120 to cell surface CD4, viral and target cell membranes fuse by a mechanism that is poorly understood, resulting in the introduction of the viral capsid into the target cell cytoplasm (4).

Mature CD4 has a relative molecular mass (Mr) of 55 kDa and consists of an N-terminal 372-amino acid extracellular domain containing four tandem immunoglobulin-like regions (V1-V4), followed by a 23-amino acid transmembrane domain and a 38-amino acid cytoplasmic segment (5, 6). In experiments using truncated sCD4 proteins, it has been shown that the determinants for high-affinity binding to HIV-1 gp120 lie solely within the N-terminal immunoglobulin-like domain (V1) (7-9). Mutational analysis of V1 has defined a discrete binding site (residues 38-52) that comprises a region structurally homologous to the second complementarity-determining region (CDR2) of immunoglobulin genes (9).

The production of large quantities of sCD4 has permitted a structural analysis of the two N-terminal immunoglobulin-like domains (V1V2). The structure determined at 2.3

angstrom resolution reveals that the molecule has two tightly-associated domains, each of which contains the immunoglobulin-fold connected by a continuous beta strand. The putative binding sites for monoclonal antibodies, class II major histocompatibility complex (MHC) molecules, and HIV-1 gp120, as determined by mutational analyses, map on the molecular surface (10, 11).

The HIV-1 envelope gene <u>env</u> encodes an envelope glycoprotein precursor, gp160, which is cleaved by cellular proteases before transport to the plasma membrane to yield gp120 and gp41. The membrane-spanning glycoprotein, gp41, is non-covalently associated with gp120, a purely extracellular glycoprotein. The mature gp120 molecule is heavily glycosylated (approximately 24 N-linked oligosaccharides), contains approximately 480 amino acid residues with 9 intra-chain disulfide bonds (12), and projects from the viral membrane as a dimeric or multimeric molecule (13).

Mutational studies of HIV-1 gp120 have delineated important functional regions of the molecule. The regions of gp120 that interact with gp41 map primarily to the N- and C-termini (14). The predominant strain-specific neutralizing epitope on gp120 is located in the 32-34 amino acid residue third variable loop, herein referred to as the V3 loop, which resides near the center of the gp120 sequence (15). The CD4 binding site maps to discontinuous regions of gp120 that include highly conserved or invariant amino acid residues in the second, third, and fourth conserved domains (the C2, C3, and C4 domains) of gp120 (16). It has been postulated that a small pocket formed by these conserved residues within gp120 could accommodate the CDR2 loop of CD4, a region defined by mutational analyses as important in interacting with gp120 (17).

HIV-1 gp120 not only mediates viral attachment to surface CD4 molecules, but also serves as the major target of antibodies which neutralize non-cell-associated virus and inhibit cell to cell viral transmission.

There are two major classifications of HIV-1-neutralizing antibodies: type-specific and group-common (15). Type-specific neutralizing antibodies primarily recognize linear determinants in the highly variable V3 loop of gp120. These antibodies act by inhibiting fusion between HIV-1 and the target cell membrane, and generally neutralize only a particular isolate of, or closely related strains of, HIV-1. Sequence variation within the V3 loop, as well as outside of this region, permits viruses to escape neutralization by anti-V3 loop antibodies. In contrast, group-common neutralizing antibodies primarily recognize discontinuous or conformational epitopes in gp120, and possess the ability to neutralize a diverse range of HIV-1 isolates. These broadly neutralizing antibodies often recognize a site on gp120 which overlaps the highly conserved CD4-binding site, and thus inhibits gp120-CD4 binding.

A structural relationship has been demonstrated between the V3 loop and the C4 region of gp120 which region constitutes both part of the CD4 binding site and part of the conserved neutralization epitopes. It was observed that deleting the V3 loop resulted in significantly increased binding of a panel of broadly neutralizing hMoAbs (neutralizing human monoclonal antibodies) to the CD4 binding site (18).

A major goal in AIDS vaccine development is to develop a vaccine able to protect a subject against the numerous genetic variants of HIV-1 that infect humans. Although cell-mediated immune responses might serve to control infection in HIV-1-infected individuals, several lines of

evidence demonstrate that protection against infection is mainly mediated by neutralizing antibodies directed against gp120. Early experiments showed that immunization of chimpanzees with recombinant gp120 induced a protective immune response against challenge with the homologous HIV-1 strain (17). This protection correlated with the presence of high-titer neutralizing antibodies against the V3 loop of gp120. In addition, passive immunization of chimpanzees with a V3-loop neutralizing monoclonal antibody resulted in protection against challenge with the homologous HIV-1 strain (19). Although protection against challenge was demonstrated in these two experiments, recent studies have questioned the clinical relevance of these findings. For example, these neutralizing antibodies recognize the V3 loop determinants of a single strain, and not conserved or discontinuous epitopes. Thus, these antibodies lack the ability to neutralize the broad spectrum of HIV-1 strains present in an HIV-1 population. Furthermore, the challenge virus was the homologous HIV-1 laboratory adapted LAI (HTLV-IIIB) strain and not one of the primary isolates that contain considerable gp120 sequence heterogeneity. Since these experiments showed that gp120 subunit vaccination induces an immune response effective against only the homogeneous HIV-1 strain used as an antigen, it is unlikely that the vaccination regimens used in these studies would be useful in humans.

Individuals infected by HIV-1 typically develop antibodies that neutralize the virus <u>in vitro</u>, and neutralization titers decrease with disease progression (19). Analysis of sera from HIV-1-infected humans indicates that type-specific neutralizing antibodies appear early in infection. Later in the course of infection, a more broadly neutralizing antibody response develops. However this antibody response is of significantly lower titer and/or affinity.

Fractionation studies of HIV-1 antibody-positive human sera reveal that the type-specific neutralizing activity is primarily directed against linear determinants in the V3 loop of gp120 (20). There was no correlation found among antibodies between the ability to neutralize divergent HIV-1 isolates and reactivity to the V3 loop of these isolates. In contrast, the broadly neutralizing antibodies present in HIV-1 antibody-positive human sera primarily recognize discontinuous epitopes in gp120 which overlap the CD4-binding site and block gp120-CD4 binding. In other words, the broadly neutralizing activity of neutralizing antibodies is not merely the result of additive anti-V3 loop reactivities against diverse HIV-1 isolates which appear during infection.

Recently, several groups have generated human monoclonal antibodies (hMoAbs) derived from HIV-1 infected individuals which possess type-specific or group-common neutralizing activities (17). The type-specific neutralizing hMoAbs were found to recognize linear determinants in the V3 loop of gp120. In contrast, the group-common neutralizing hMoAbs generally recognize discontinuous epitopes which overlap the CD4-binding site and block gp120-CD4 binding.

The V3 loop is a highly immunodominant region of gp120 which partially interacts with the CD4-binding region. The presence of the V3 loop region on gp120 may skew the humoral immune response away from producing antibodies which specifically bind to the CD4-binding domain of gp120. Furthermore, the advantages of removing the V3 loop to expose the CD4-binding domain of gp120 to the immune system would be countered by the fact that the exposed CD4-binding site would still have a high affinity for cell surface CD4. In other words, a mutant gp120 protein missing only the V3 loop would quickly bind to CD4+ cells and would thus be

hampered in generating an immune response against the exposed CD4-binding site.

The subject invention provides a mutant HIV-1 gp120 envelope glycoprotein which overcomes both the problems of V3 loop immunodominance and of the high affinity to CD4. The subject invention further provides vaccines comprising the mutant HIV-1 gp120 envelope glycoprotein, antibodies which specifically bind to the CD4-binding site of HIV-1 gp120 envelope glycoprotein, pharmaceutical compositions comprising these antibodies, and methods of using these vaccines and compositions to treat or prevent HIV-1 infection.

Summary of the Invention

The subject invention provides a recombinant nucleic acid molecule which encodes a mutant HIV-1 gp120 envelope glycoprotein comprising a V3 loop deletion and a C4 $\text{domain}_{(W\text{-}>X)}$ point mutation, wherein X is an amino acid residue other than tryptophan. In the preferred embodiment, X is a valine residue.

In one embodiment, the nucleic acid molecule is a DNA molecule. The DNA molecule may be a plasmid. In one embodiment, the plasmid comprises the sequence of the plasmid designated PPI4-tPA.

In one embodiment, the C4 domain is an HIV-1_{LAI} gp120 envelope glycoprotein C4 domain. The mutant HIV-1 gp120 envelope glycoprotein may be a mutant HIV-1_{LAI} gp120 envelope glycoprotein.

In another embodiment, the C4 domain is an $\text{HIV-1}_{JR\text{-}FL}$ gp120 envelope glycoprotein C4 domain. The mutant HIV-1 gp120 envelope glycoprotein may be a mutant $\text{HIV-1}_{JR\text{-}FL}$ gp120 envelope glycoprotein.

The subject invention also provides the mutant HIV-1 gp120 envelope glycoprotein encoded by the recombinant nucleic acid molecule of the subject invention.

The subject invention further provides a vaccine which comprises a therapeutically effective amount of the mutant HIV-1 gp120 envelope glycoprotein of the subject invention, and an adjuvant.

The subject invention further provides a method of treating

an HIV-1-infected subject, which comprises immunizing the HIV-1-infected subject with the vaccine of the subject invention, thereby treating the HIV-1-infected subject.

The subject invention further provides a vaccine which comprises a prophylactically effective amount of the mutant HIV-1 gp120 envelope glycoprotein of the subject invention, and an adjuvant.

The subject invention further provides a method of reducing the likelihood of an HIV-1-exposed subject's becoming infected with HIV-1, which comprises immunizing the HIV-1-exposed subject with the vaccine of the subject invention, thereby reducing the likelihood of the HIV-1-exposed subject's becoming infected with HIV-1.

The subject invention further provides a method of reducing the likelihood of a non-HIV-1-exposed subject's becoming infected with HIV-1, which comprises immunizing the non-HIV-1-exposed subject with the vaccine of the subject invention, thereby reducing the likelihood of the non-HIV-1-exposed subject's becoming infected with HIV-1.

The subject invention further provides a method of obtaining partially purified antibodies which specifically bind to the CD4-binding domain of HIV-1 gp120 envelope glycoprotein, which method comprises (a) immunizing a non-HIV-1-exposed subject with the vaccine of the subject invention, (b) recovering from the immunized subject serum comprising said antibodies, and (c) partially purifying said antibodies, thereby obtaining partially purified antibodies which specifically bind to the CD4-binding domain of HIV-1 gp120 envelope glycoprotein. In the preferred embodiment, the subject is a human.

The subject invention further provides the partially purified antibodies produced by the method of the subject invention.

The subject invention further provides a pharmaceutical composition, which comprises a therapeutically effective amount of the partially purified antibodies of the subject invention, and a pharmaceutically acceptable carrier.

The subject invention further provides a method of treating an HIV-1-infected subject, which comprises administering to the subject a dose of the pharmaceutical composition of the subject invention effective to reduce the population of HIV-1-infected cells in the HIV-1-infected subject, thereby treating the HIV-1-infected subject.

The subject invention further provides a method of treating an HIV-1-infected subject, which comprises administering to the subject a dose of the pharmaceutical composition of the subject invention effective to reduce the population of HIV-1 in the HIV-1-infected subject, thereby treating the HIV-1-infected subject.

The subject invention further provides a composition which comprises a prophylactically effective amount of the partially purified antibodies of the subject invention, and a pharmaceutically acceptable carrier.

The subject invention further provides a method of reducing the likelihood of an HIV-1-exposed subject's becoming infected with HIV-1, which comprises administering to the HIV-1-exposed subject a dose of the composition of the subject invention effective to reduce the population of HIV-1 in the HIV-1-exposed subject, thereby reducing the likelihood of the subject's becoming infected with HIV-1.

In one embodiment, the subject is a medical practitioner. In another embodiment, the subject is a newborn infant.

Finally, the subject invention provides a method of reducing the likelihood of a non-HIV-1-exposed subject's becoming infected with HIV-1 as a result of exposure thereto during an incident wherein there is an increased risk of exposure to HIV-1, which comprises administering to the subject immediately prior to the incident a dose of the composition of the subject invention effective to reduce the population of HIV-1 to which the subject is exposed during the incident, thereby reducing the likelihood of the subject's becoming infected with HIV-1. In one embodiment, the subject is a medical practitioner.

Brief Description of the Figures

Figure 1

gp120 structure. Shown is a box diagram of HIV-1 gp120 depicting the boundaries of the five constant domains (C1-C5) and the five variable domains (V1-V5). The amino acid residue numbering above the box begins at the initiator methionine found at the beginning of the signal sequence (S) and is approximated based on a consensus of all known HIV-1 gp120 amino acid sequences. Also shown are the C4 domain amino acid sequences of HIV-1 strains LAI and JR-FL. Above the C4 domain sequences are indicated two mutations that reduce gp120 binding to cell surface CD4; tryptophan to valine and aspartate to alanine.

Figure 2

PPI4-tPA-gp120$_{LAI}$. Expression vector with the HIV-1$_{LAI}$ gp120 gene fused to the CMV MIE promoter, and the tPA signal sequence replacing the HIV-1 gp120 signal sequence. Abbreviations: CMV MIE = cytomegalovirus major immediate early, E = enhancer, P = promoter, EXA = Exon A, INA = Intron A, EXB = Exon B, tPA ss = human tissue plasminogen activator signal sequence, gp120 = glycoprotein 120, BGH = bovine growth hormone, AMP = ampicillin resistance gene, and DHFR = dihydrofolate reductase gene.

Figure 3

CMV MIE promoter fused to tPA-gp120$_{LAI}$. The nucleotide sequence of the CMV MIE promoter/enhancer region is shown fused to the HIV-1$_{LAI}$ gp120 gene that contains the tPA signal sequence. The numbering of nucleotide sequence begins with the HincII site and the numbering of the amino acid sequence begins with the first methionine found in the tPA signal sequence. The tPA signal sequence is fused in-frame to Thr$_{31}$

of gp120, the first amino acid found in mature gp120. The signal sequence is shown in bold as are various landmark restriction sites used for cloning as discussed in the text. The locations of Exon A, Intron A, Exon B and the transcription start site and the signal cleavage site are indicated.

Figure 4

Transient expression of gp120. Autoradiograph of ^{35}S-labeled supernatants from COS cell transfectants, immunoprecipitated with a CD4-immunoglobulin-Protein A-Sepharose complex, and run on a reducing 10% SDS-PAGE gel. The plasmids used for transfection were: Lane 1: Mock transfected cells; lane 2: a vector encoding a CD4-immunoglobulin chimera as a positive transfection control; lane 3: PPI4-tPA-gp120$_{LAI}$; and lane 4: PPI4-tPA-gp120$_{JR-FL}$. Positions of molecular weight markers are indicated.

Figure 5

Determination of gp120 concentration by ELISA. Panel A: Concentrations of gp120 in media of CHO cell lines, stably transfected with PPI4-tPA-gp120$_{LAI}$, determined by ELISA. Panel B: A standard curve was established using known amounts of gp120.

Figure 6

Expression of gp120 in stably transfected CHO cells. Autoradiograph of ^{35}S-labeled supernatants from stable CHO cell lines, immunoprecipitated with a CD4-immunoglobulin-Protein A-Sepharose complex, and run on a reducing 10% SDS-PAGE gel. Lane 1: clone 9; lane 2: clone 13; lane 3: clone 6; lane 4: Clone 5. Positions of molecular weight markers are indicated.

Figure 7

tPA-gp120$_{JR-FL}$. The nucleotide and deduced amino acid sequence of the tPA signal sequence fused to HIV-1$_{JR-FL}$ gp120 is shown. The NarI and NotI restriction endonuclease sites used for cloning are shown in bold. The predicted site of cleavage by signal peptidase between Arg$_{35}$ and Val$_{36}$ is indicated.

Figure 8

tPA-gp120$_{LAI}$-V3$^{(-)}$. The nucleotide and deduced amino acid sequence of the tPA signal sequence fused to HIV-1$_{LAI}$ gp120 with the V3 loop deleted and replaced with the pentapeptide TGAGH is shown. The V3 loop replacement and the NarI and NotI restriction endonuclease sites used for cloning are shown in bold. The predicted site of cleavage by signal peptidase between Arg$_{35}$ and Thr$_{36}$ is indicated.

Figure 9

tPA-gp120$_{JR-FL}$-V3$^{(-)}$. The nucleotide and deduced amino acid sequence of the tPA signal sequence fused to HIV-1$_{JR-FL}$ gp120 with the V3 loop deleted and replaced with the pentapeptide TGAGH is shown. The V3 loop replacement and the NarI and NotI restriction endonuclease sites used for cloning are shown in bold. The predicted site of cleavage by signal peptidase between Arg$_{35}$ and Val$_{36}$ is indicated.

Figure 10

tPA-gp120$_{LAI}$-V3$^{(-)}$-CD4$^{(-)}$. Shown is the nucleotide and deduced amino acid sequence of the tPA signal sequence fused to HIV-1$_{LAI}$ gp120, with the V3 loop deleted and replaced with the pentapeptide TGAGH, and Trp$_{403}$ mutated to Val. The mutations and the NarI and NotI restriction endonuclease sites used for cloning are shown in bold. The predicted site of cleavage by signal peptidase between Arg$_{35}$ and Thr$_{36}$ is indicated.

Figure 11

tPA-gp120$_{JR-FL}$-V3$^{(-)}$-CD4$^{(-)}$. Shown is the nucleotide and deduced amino acid sequence of the tPA signal sequence fused to HIV-1$_{JR-FL}$ gp120, with the V3 loop deleted and replaced with the pentapeptide TGAGH, and Trp$_{396}$ mutated to Val. The mutations and the NarI and NotI restriction endonuclease sites used for cloning are shown in bold. The predicted site of cleavage by signal peptidase between Arg$_{35}$ and Val$_{36}$ is indicated.

Figure 12

tPA-gp120$_{LAI}$-CD4$^{(-)}$. Shown is the nucleotide and deduced amino acid sequence of the tPA signal sequence fused to HIV-1$_{LAI}$ gp120. The Trp$_{437}$ to Val CD4 binding mutation, the NarI and NotI restriction endonuclease sites used for cloning, and the predicted site of cleavage by signal peptidase between Arg$_{35}$ and Thr$_{36}$ are shown in bold.

Figure 13

tPA-gp120$_{JR-FL}$-CD4$^{(-)}$. Shown is the nucleotide and deduced amino acid sequence of the tPA signal sequence fused to HIV-1$_{JR-FL}$ gp120. The Trp$_{424}$ to Val CD4 binding mutation, the NarI and NotI restriction endonuclease sites used for cloning and the predicted cleavage by signal peptidase between Arg$_{35}$ and Val$_{36}$ are shown in bold.

Figure 14

Expression of gp120 in stably transfected CHO cells. Autoradiograph of super ^{35}S-labeled supernatants from stable CHO cell lines, immunoprecipitated with MoAb F105-Protein A-Sepharose complex, and run on a reducing 10% SDS-PAGE gel. Panel A: Lane 1: tPA-gp120$_{LAI}$ CHO cells; lane 2: tPA-gp120$_{LAI}$-V3$^{(-)}$ CHO cells; lane 3: tPA-gp120$_{LAI}$-V3$^{(-)}$-CD4$^{(-)}$ CHO cells. Panel B: Lane 1: tPA-gp120$_{JR-FL}$ CHO cells; lane 2: tPA-gp120$_{JR-FL}$-V3$^{(-)}$

CHO cells; lane 3: tPA-gp120$_{JR-FL}$-V3$^{(-)}$-CD4$^{(-)}$ CHO cells. Positions of molecular weight markers are indicated.

Figure 15

Purified gp120 proteins.

Silver stained 10% SDS-PAGE gel with a sample of purified gp120 proteins. Panel A: Lane 1: tPA-gp120$_{LAI}$ CHO cells; lane 2: tPA-gp120$_{LAI}$-V3$^{(-)}$ CHO cells; lane 3: tPA-gp120$_{LAI}$-V3$^{(-)}$-CD4$^{(-)}$ CHO cells. Panel B: Lane 1: tPA-gp120$_{JR-FL}$ CHO cells; lane 2: tPA-gp120$_{JR-FL}$-V3$^{(-)}$ CHO cells; lane 3: tPA-gp120$_{JR-FL}$-V3$^{(-)}$-CD4$^{(-)}$ CHO cells. Positions of molecular weight markers are indicated.

Figure 16

Analysis of binding of recombinant mutant gp120 to cell surface human CD4 by FACS.

Plate 1. DG44 cells, a subclone of CHO cells which lack expression of the human CD4 protein, were used as control. Increasing concentrations of HIV-1 gp120$_{LAI}$ did not show an increase in specific fluoresence when compared to background. Plate 2. DG44 #3 cells are a CHO cell line transfected with the cDNA clone encoding the human CD4 protein. Increasing concentrations of HIV-1 gp120$_{LAI}$ show a dramatic increase (or shift) in fluoresence. Plate 3. Similar to Plate 2 but the HIV-1 gp120$_{LAI}$-V3$^{(-)}$ protein was added. Again a large shift indicating binding to the DG44 #3 cells was seen. Plate 4. DG44 #3 cells were incubated with either HIV-1 gp120$_{LAI}$-V3$^{(-)}$-CD4$^{(-)}$ protein or MoAb OKT4A an antibody with high affinity for human CD4. Only OKT4A bound to the cells.

Detailed Description of the Invention

The plasmids designated PPI4-tPA-gp120$_{LAI}$ and PPI4-tPA-gp120$_{JR-FL}$ were deposited pursuant to, and in satisfaction of, the requirements of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852 under ATCC Accession Nos. 75431 and 75432, respectively. The plasmids PPI4-tPA-gp120$_{LAI}$ and PPI4-tPA-gp120$_{JR-FL}$ were deposited with the ATCC on March 12, 1993.

The subject invention provides a recombinant nucleic acid molecule which encodes a mutant HIV-1 gp120 envelope glycoprotein comprising a V3 loop deletion and a C4 domain$_{(W->X)}$ point mutation, wherein X is an amino acid residue other than tryptophan. In the preferred embodiment, X is a valine residue.

In one embodiment, the nucleic acid molecule is a DNA molecule. The DNA molecule may be a plasmid. In one embodiment, the plasmid comprises the sequence of the plasmid designated PPI4-tPA.

The V3 loop of HIV-1 gp120 envelope glycoprotein is shown in Figure 1. The V3 loop is demarcated by cysteine residues at both its N- and C-termini. As used herein, a V3 loop deletion means a deletion of one or more amino acid residues between the terminal cysteine residues, with the proviso that there must be three or more amino acid residues situated between the two terminal cysteine residues in a V3 loop deletion. These three or more amino acid residues may either be residues originally present in the V3 loop, or exogenous residues. For example, as shown in the

Experimental Details section *infra*, the pentapeptide TGAGH is situated between the two terminal cysteine residues. Variations in the size of the V3 loop deletion illustrated herein are tolerable without affecting the overall structure of the mutant HIV-1 gp120 envelope glycoprotein, as is well known to those skilled in the art.

As used herein, "C4 domain" means the HIV-1 gp120 envelope glycoprotein C4 domain having the following consensus sequence:

$$X_1X_2X_3CX_4IX_5X_6X_7X_8X_9X_{10}\mathbf{W}X_{11}X_{12}X_{13}X_{14}X_{15}AX_{16}YX_{17}X_{18}\text{-}PX_{19}X_{20}X_{21}X_{22}X_{23}X_{24}X_{25}X_{26}SX_{27}X_{28}TGX_{29}X_{30}X_{31}X_{32}RX_{33}GX_{34},$$

wherein X_1 = T, I, V, K or R; X_2 = L, I or H; X_3 = P, Q, L or T; X_4 = R, K or G; X_5 = K or E; X_6 = Q or E; X_7 = F, I or V; X_8 = I, V or M; X_9 = N, R or K; X_{10} = M, R, L or T; X_{11} = Q, R or V; X_{12} = E, K, G, R, V or A; X_{13} = V, T, A or G; X_{14} = G or E; X_{15} = K, R, E, or Q; X_{16} = M, V, I or L; X_{17} = A, T or D; X_{18} = P or L; X_{19} = I or F; X_{20} = S, R, G, K, N, A, E or Q; X_{21} = G or R; X_{22} = Q, L, P, N, K, V, T, E or I; X_{23} = I, V or L; X_{24} = R, K, S, N, G, I, T, E or I; X_{25} = C or R; X_{26} = S, L, I, T, P, E, V, K, D or N; X_{27} = N, K or L; X_{28} = I or V; X_{29} = L, P or I; X_{30} = L or I; X_{31} = L or I; X_{32} = T, A, I, V or E; X_{33} = D or E; X_{34} = G or V.

The C4 domain consensus sequence is based on existing C4 domain sequence information from various HIV-1 strains, and thus is not necessarily an exhaustive consensus sequence. The conserved tryptophan residue shown in bold after residue X_{10} is the only conserved tryptophan residue in the C4 domain. As used herein, a C4 domain$_{(W\text{->}X)}$ point mutation is a mutation of the above-identified conserved C4 domain tryptophan residue to an amino acid residue other than

tryptophan. For example, a C4 domain$_{(W\to V)}$ point mutation is a mutation of the conserved C4 domain tryptophan residue to a valine residue.

In one embodiment, the C4 domain is an HIV-1_{LAI} gp120 envelope glycoprotein C4 domain. The sequence of the HIV-1_{LAI} gp120 C4 domain is: TLPCRIKQFINMWQEVGKAMYAPPISGQIRCS-SNITGLLLTRDGG. The mutant HIV-1 gp120 envelope glycoprotein may be a mutant HIV-1_{LAI} gp120 envelope glycoprotein.

In another embodiment, the C4 domain is an HIV-1_{JR-FL} gp120 envelope glycoprotein C4 domain. The sequence of the HIV-1_{JR-FL} gp120 C4 domain is: TLPCRIKQIINMWQEVGKAMYAPPIRGQIRCS-SNITGLLLTRDGG. The mutant HIV-1 gp120 envelope glycoprotein may be a mutant HIV-1_{JR-FL} gp120 envelope glycoprotein.

HIV-1_{LAI} is a laboratory-adapted strain that is tropic for phytohemagglutinin (PHA)-stimulated peripheral blood lymphocytes (PBLs) and immortalized human T-cell lines. In contrast, HIV-1_{JR-FL} was isolated from brain tissue taken at autopsy that was co-cultured with lectin-activated normal human PBLs. HIV-1_{JR-FL} is tropic for PHA-stimulated PBLs and blood-derived macrophages but will not replicate in transformed T-cell lines. Mutant HIV-1 gp120 envelope glycoproteins derived from a clinical isolate of HIV-1 such as JR-FL may possess new or different epitopes compared to the laboratory-adapted HIV-1 strains that are beneficial for successful vaccination. Although only the HIV-1_{LAI} and HIV-1_{JR-FL} strains are used herein to generate the mutant HIV-1 gp120 envelope glycoproteins of the subject invention, other HIV-1 strain could be substituted in their place as is well known to those skilled in the art.

The V1 and V2 variable regions of gp120 are unnecessary for

CD4 binding (21). Therefore the mutant HIV-1 gp120 envelope glycoprotein of this invention can either include or exclude the V1 and V2 variable regions.

The subject invention additionally provides a recombinant nucleic acid molecule which encodes a mutant HIV-1 gp120 envelope glycoprotein comprising a V3 loop deletion and a C4 domain$_{(Asp\rightarrow X)}$ point mutation, wherein the aspartate residue is between amino acid residues X_{15} and X_{16} in the C4 consensus sequence, and X is an amino acid residue other than aspartate or glutamate. In the preferred embodiment, X is an alanine residue.

The subject invention additionally provides a recombinant nucleic acid molecule which encodes a mutant HIV-1 gp120 envelope glycoprotein comprising a V3 loop deletion and a C4 domain$_{(Glu\rightarrow X)}$ point mutation, wherein the glutamate residue is between amino acid residues X_{15} and X_{16} in the C4 consensus sequence, and X is an amino acid residue other than aspartate or glutamate. In the preferred embodiment, X is an alanine residue.

The subject invention additionally provides a recombinant nucleic acid molecule which encodes a mutant HIV-1_{LAI} gp120 envelope glycoprotein comprising a V3 loop deletion and a C3 domain$_{(asp378\rightarrow X)}$ point mutation, wherein X is an amino acid residue other than aspartate or glutamate. In the preferred embodiment, X is a lysine residue.

The subject invention additionally provides a recombinant nucleic acid molecule which encodes a mutant HIV-$1_{JR\text{-}FL}$ gp120 envelope glycoprotein comprising a V3 loop deletion and a C3 domain$_{(asp369\rightarrow X)}$ point mutation, wherein X is an amino acid residue other than aspartate or glutamate. In the preferred

embodiment, X is a lysine residue.

The subject invention additionally provides a recombinant nucleic acid molecule which encodes a mutant HIV-1_{LAI} gp120 envelope glycoprotein comprising a V3 loop deletion and a C3 $domain_{(glu380->X)}$ point mutation, wherein X is an amino acid residue other than glutamate. In the preferred embodiment, X is a glutamine residue.

The subject invention additionally provides a recombinant nucleic acid molecule which encodes a mutant HIV-1_{JR-FL} gp120 envelope glycoprotein comprising a V3 loop deletion and a C3 $domain_{(glu371->X)}$ point mutation, wherein X is an amino acid residue other than glutamate. In the preferred embodiment, X is a glutamine residue.

The subject invention additionally provides a recombinant nucleic acid molecule which encodes a mutant HIV-1_{LAI} gp120 envelope glycoprotein comprising a V3 loop deletion and a C2 $domain_{(thr267->X)}$ point mutation, wherein X is an amino acid residue other than threonine. In the preferred embodiment, X is an arginine residue.

The subject invention additionally provides a recombinant nucleic acid molecule which encodes a mutant HIV-1_{JR-FL} gp120 envelope glycoprotein comprising a V3 loop deletion and a C2 $domain_{(thr260->X)}$ point mutation, wherein X is an amino acid residue other than threonine. In the preferred embodiment, X is an arginine residue.

The subject invention additionally provides a recombinant nucleic acid molecule which encodes a mutant HIV-1 gp120 envelope glycoprotein comprising (a) a V3 loop deletion, or (b) a one of the C2, C3 or C4 domain point mutations

discussed _supra_.

The point mutations in the recombinant nucleic acid molecules described _supra_ are selected based on their ability to reduce the affinity of the mutant gp120 glycoprotein encoded thereby for CD4. As used herein, the term "reduce the affinity" means to reduce the affinity by at least two-fold.

One skilled in the art would know how to make recombinant nucleic acid molecules which encode mutant HIV-1 gp120 envelope glycoproteins comprising a V3 loop deletion and the specific C2, C3 or C4 domain point mutations corresponding to those mutations exemplified in the HIV-1_{JR-FL} and HIV-1_{LAI} strains, _supra_. Furthermore, one skilled in the art would know how to use these recombinant nucleic acid molecules to obtain the proteins encoded thereby, and practice the therapeutic and prophylactic methods of using same, as described herein for the recombinant nucleic acid molecule which encodes a mutant HIV-1 gp120 envelope glycoprotein comprising a V3 loop deletion and a C4 domain$_{(W->X)}$ point mutation.

The subject invention also provides the mutant HIV-1 gp120 envelope glycoprotein encoded by the recombinant nucleic acid molecule of the subject invention.

In accordance with the invention, numerous vector systems for expression of the mutant HIV-1 gp120 envelope glycoprotein may be employed. For example, one class of vectors utilizes DNA elements which are derived from animal viruses such as bovine papilloma virus, polyoma virus, adenovirus, vaccinia virus, baculovirus, retroviruses (RSV, MMTV or MoMLV), Semliki Forest virus or SV40 virus.

Additionally, cells which have stably integrated the DNA into their chromosomes may be selected by introducing one or more markers which allow for the selection of transfected host cells. The marker may provide, for example, prototropy to an auxotrophic host, biocide resistance, (e.g., antibiotics) or resistance to heavy metals such as copper or the like. The selectable marker gene can be either directly linked to the DNA sequences to be expressed, or introduced into the same cell by cotransformation. Additional elements may also be needed for optimal synthesis of mRNA. These elements may include splice signals, as well as transcriptional promoters, enhancers, and termination signals. The cDNA expression vectors incorporating such elements include those described by Okayama (22).

The vectors used in the subject invention are designed to express high levels of mutant HIV-1 gp120 envelope glycoproteins in cultured eukaryotic cells as well as efficiently secrete these proteins into the culture medium. The targeting of the mutant HIV-1 gp120 envelope glycoproteins into the culture medium is accomplished by fusing in-frame to the mature N-terminus of the mutant HIV-1 gp120 envelope glycoprotein the tissue plasminogen activator (tPA) prepro-signal sequence.

The mutant HIV-1 gp120 envelope glycoprotein may be produced by a) transfecting a mammalian cell with an expression vector for producing mutant HIV-1 gp120 envelope glycoprotein; b) culturing the resulting transfected mammalian cell under conditions such that mutant HIV-1 gp120 envelope glycoprotein is produced; and c) recovering the mutant HIV-1 gp120 envelope glycoprotein so produced.

Once the expression vector or DNA sequence containing the constructs has been prepared for expression, the expression

vectors may be transfected or introduced into an appropriate mammalian cell host. Various techniques may be employed to achieve this, such as, for example, protoplast fusion, calcium phosphate precipitation, electroporation or other conventional techniques. In the case of protoplast fusion, the cells are grown in media and screened for the appropriate activity. Expression of the gene encoding a mutant HIV-1 gp120 envelope glycoprotein results in production of the mutant glycoprotein.

Methods and conditions for culturing the resulting transfected cells and for recovering the mutant HIV-1 gp120 envelope glycoprotein so produced are well known to those skilled in the art, and may be varied or optimized depending upon the specific expression vector and mammalian host cell employed.

In accordance with the claimed invention, the preferred host cells for expressing the mutant HIV-1 gp120 envelope glycoprotein of this invention are mammalian cell lines. Mammalian cell lines include, for example, monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line 293; baby hamster kidney cells (BHK); Chinese hamster ovary-cells-DHFR (CHO); Chinese hamster ovary-cells DHFR$^-$ (DXB11); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); mouse cell line (C127); and myeloma cell lines.

Other eukaryotic expression systems utilizing non-mammalian vector/cell line combinations can be used to produce the mutant HIV-1 gp120 envelope glycoproteins. These include, but are not limited to, baculovirus vector/insect cell

expression systems and yeast shuttle vector/yeast cell expression systems.

Methods and conditions for purifying mutant HIV-1 gp120 envelope glycoproteins from the culture media are provided in the invention, but it should be recognized that these procedures can be varied or optimized as is well known to those skilled in the art.

The subject invention further provides a vaccine which comprises a therapeutically effective amount of the mutant HIV-1 gp120 envelope glycoprotein of the subject invention, and an adjuvant.

A therapeutically effective amount of the mutant HIV-1 gp120 envelope glycoprotein may be determined according to methods well known to those skilled in the art.

As used herein, adjuvants include, but are not limited to, alum, Freund's incomplete adjuvant (FIA), Saponin, Quil A, Monophosphoryl lipid A (MPL), and nonionic block copolymers (SAF) such as L-121 (Pluronic; Syntex SAF). In the preferred embodiment, the adjuvant is alum, especially in the form of a thixotropic, viscous, and homogeneous aluminum hydroxide gel. The vaccine of the subject invention may be administered as an oil in water emulsion. Methods of combining adjuvants with antigens are well known to those skilled in the art.

The subject invention further provides a method of treating an HIV-1-infected subject, which comprises immunizing the HIV-1-infected subject with the vaccine of the subject invention, thereby treating the HIV-1-infected subject.

As used herein, treating an HIV-1-infected subject with the

vaccine of the subject invention means reducing in the subject either the population of HIV-1 or HIV-1-infected cells, or ameliorating the progression of an HIV-1-related disorder in the subject.

As used herein, an "HIV-infected subject" means an individual having at least one of his own cells invaded by HIV-1.

As used herein, "immunizing" means administering a primary dose of the vaccine to a subject, followed after a suitable period of time by one or more subsequent administrations of the vaccine, so as to generate in the subject an immune response against the CD4-binding region of the mutant HIV-1 gp120 envelope glycoprotein in the vaccine. A suitable period of time between administrations of the vaccine may readily be determined by one skilled in the art, and is usually in the order of several weeks to months.

In the preferred embodiment, the dose of vaccine administered is an amount sufficient to deliver to the subject between 10ug and 1mg of the mutant HIV-1 gp120 envelope glycoprotein.

The subject invention further provides a vaccine which comprises a prophylactically effective amount of the mutant HIV-1 gp120 envelope glycoprotein of the subject invention, and an adjuvant.

A prophylactically effective amount of the mutant HIV-1 gp120 envelope glycoprotein may be determined according to methods well known to those skilled in the art.

The subject invention further provides a method of reducing the likelihood of an HIV-1-exposed subject's becoming

infected with HIV-1, which comprises immunizing the HIV-1-exposed subject with the vaccine of the subject invention, thereby reducing the likelihood of the HIV-1-exposed subject's becoming infected with HIV-1.

As used herein, the subject's becoming infected with HIV-1 means the invasion of the subject's own cells by HIV-1.

As used herein, reducing the likelihood of a subject's becoming infected with HIV-1 means reducing the likelihood of the subject's becoming infected with HIV-1 by at least two-fold. For example, if a subject has a 1% chance of becoming infected with HIV-1, a two-fold reduction in the likelihood of the subject's becoming infected with HIV-1 would result in the subject's having a 0.5% chance of becoming infected with HIV-1. In the preferred embodiment of this invention, reducing the likelihood of the subject's becoming infected with HIV-1 means reducing the likelihood of the subject's becoming infected with HIV-1 by at least ten-fold.

As used herein, an HIV-1-exposed subject is a subject who has HIV-1 present in his body, but has not yet become HIV-1-infected.

The subject invention further provides a method of reducing the likelihood of a non-HIV-1-exposed subject's becoming infected with HIV-1, which comprises immunizing the non-HIV-1-exposed subject with the vaccine of the subject invention, thereby reducing the likelihood of the non-HIV-1-exposed subject's becoming infected with HIV-1.

As used herein, a non-HIV-1-exposed subject is a subject who does not have HIV-1 present in his body.

The subject invention further provides a method of obtaining partially purified antibodies which specifically bind to the CD4-binding domain of HIV-1 gp120 envelope glycoprotein, which method comprises (a) immunizing a non-HIV-1-exposed subject with the vaccine of the subject invention, (b) recovering from the immunized subject serum comprising said antibodies, and (c) partially purifying said antibodies, thereby obtaining partially purified antibodies which specifically bind to the CD4-binding domain of HIV-1 gp120 envelope glycoprotein. In the preferred embodiment, the subject is a human.

As used herein, partially purified antibodies means a composition which comprises antibodies which specifically bind to the CD4-binding domain of HIV-1 gp120 envelope glycoprotein, and consists of fewer protein impurities than does the serum from which the anti-CD4-binding domain antibodies are derived. A protein impurity means a protein other than the anti-CD4-binding domain antibodies. For example, the partially purified antibodies might be an IgG preparation.

Methods of recovering serum from a subject are well known to those skilled in the art. Methods of partially purifying antibodies are also well known to those skilled in the art, and include, by way of example, filtration, ion exchange chromatography, and precipitation.

In one embodiment, the partially purified antibodies comprise an immune globulin (IG) preparation. IG can be purified from serum by a two-step process. Initially, serum is fractionated by the cold ethanol method of Cohn, et al. (29). Cohn Fraction II has as its main protein component IgG immunoglobulin present as monomers, dimers and aggregates. Fraction II is then purified to produce IVIG

(immune globulin intravenous) using a variety of purification methods which include, for example, ion exchange, DEAE chromatography, acid pH 4.25 diafiltration, PEG precipitation or Pepsin treatment. The final product is stabilized (e.g., glucose + NaCl) and the final IgG concentration is fixed at between about 3% and about 6%.

The subject invention further provides the partially purified antibodies produced by the method of the subject invention.

The subject invention further provides a pharmaceutical composition, which comprises a therapeutically effective amount of the partially purified antibodies of the subject invention, and a pharmaceutically acceptable carrier.

A therapeutically effective amount of the partially purified antibodies of the subject invention may be determined according to methods well known to those skilled in the art.

Pharmaceutically acceptable carriers are well known to those skilled in the art and include, but are not limited to, 0.01-0.1M and preferably 0.05M phosphate buffer or 0.8% saline. Additionally, such pharmaceutically acceptable carriers may be aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers such as

those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, antimicrobials, antioxidants, chelating agents, inert gases and the like.

The subject invention further provides a method of treating an HIV-1-infected subject, which comprises administering to the subject a dose of the pharmaceutical composition of the subject invention effective to reduce the population of HIV-1-infected cells in the HIV-1-infected subject, thereby treating the HIV-1-infected subject.

As used herein, administering may be effected or performed using any of the various methods known to those skilled in the art. The administering may comprise administering intravenously. The administering may also comprise administering intramuscularly. The administering may further comprise administering subcutaneously.

The dose of the pharmaceutical composition of the subject invention effective to reduce the population of HIV-1-infected cells in the HIV-1-infected subject may be readily determined using methods well known to those skilled in the art. In the preferred embodiment, the dose is sufficient to deliver to the subject between about 10 mg/kg and 150mg/kg of protein if administered intramuscularly. In the preferred embodiment, the dose is sufficient to deliver to the subject between about 100 mg/kg and 2g/kg of protein if administered intravenously.

The subject invention further provides a method of treating an HIV-1-infected subject, which comprises administering to the subject a dose of the pharmaceutical composition of the subject invention effective to reduce the population of HIV-1 in the HIV-1-infected subject, thereby treating the HIV-1-

infected subject.

The dose of the pharmaceutical composition of the subject invention effective to reduce the population of HIV-1 in the HIV-1-infected subject may be readily determined using methods well known to those skilled in the art. In the preferred embodiment, the dose is sufficient to deliver to the subject between about 10 mg/kg and 150mg/kg of protein if administered intramuscularly. In the preferred embodiment, the dose is sufficient to deliver to the subject between about 100 mg/kg and 2g/kg of protein if administered intravenously.

The subject invention further provides a composition which comprises a prophylactically effective amount of the partially purified antibodies of the subject invention, and a pharmaceutically acceptable carrier.

A prophylactically effective amount of the partially purified antibodies of the subject invention may be determined according to methods well known to those skilled in the art.

The subject invention further provides a method of reducing the likelihood of an HIV-1-exposed subject's becoming infected with HIV-1, which comprises administering to the HIV-1-exposed subject a dose of the composition of the subject invention effective to reduce the population of HIV-1 in the HIV-1-exposed subject, thereby reducing the likelihood of the subject's becoming infected with HIV-1.

In one embodiment, the subject is a medical practitioner. The medical practitioner may be a medical practitioner exposed to an HIV-1-containing bodily fluid. As used herein, the term "medical practitioner" includes, but is in no way

limited to, doctors, dentists, surgeons, nurses, medical laboratory assistants, and students in health care programs.

In another embodiment, the subject is a newborn infant. The newborn infant may be a newborn infant born to an HIV-1-infected mother.

The dose of the composition of the subject invention effective to reduce the population of HIV-1 in the HIV-1-exposed subject may be readily determined using methods well known to those skilled in the art. In the preferred embodiment, the dose is sufficient to deliver to the subject between about 10mg/kg and 150mg/kg of protein if administered intramuscularly. In the preferred embodiment, the dose is sufficient to deliver to the subject between about 100 mg/kg and 2g/kg of protein if administered intravenously.

The vaccines and pharmaceutical compositions of the subject invention may also ameliorate the progression of an HIV-1-related disorder in a subject to whom the vaccines or pharmaceutical compositions were administered while the subject was either non-HIV-1-exposed or HIV-1-exposed, but not yet HIV-1-infected.

Finally, the subject invention provides a method of reducing the likelihood of a non-HIV-1-exposed subject's becoming infected with HIV-1 as a result of exposure thereto during an incident wherein there is an increased risk of exposure to HIV-1, which comprises administering to the subject immediately prior to the incident a dose of the composition of the subject invention effective to reduce the population of HIV-1 to which the subject is exposed during the incident, thereby reducing the likelihood of the subject's becoming infected with HIV-1. In one embodiment, the

subject is a medical practitioner.

An incident wherein there is an increased risk of exposure to HIV-1 includes, for example, receiving a blood transfusion, sexual contact with an HIV-1-infected individual, and performing a HIV-1-containing bodily fluid-exposing medical procedure.

As used herein, "immediately prior to the incident" means within one month of the incident. In the preferred embodiment, "immediately prior to the incident" means within one day of the incident.

The dose of the composition of the subject invention effective to reduce the population of HIV-1 to which the subject is exposed during the incident may be readily determined using methods well known to those skilled in the art. In the preferred embodiment, the dose is sufficient to deliver to the subject between about 10mg/kg and 150mg/kg of protein if administered intramuscularly. In the preferred embodiment, the dose is sufficient to deliver to the subject between about 100mg/kg and 2g/kg of protein if administered intravenously.

One embodiment of this invention is a method of substantially reducing the likelihood of a non-infected medical practitioner's becoming infected with HIV-1 during a bodily fluid-exposing medical procedure involving a patient, which comprises administering to the patient during a suitable time period an amount of the composition of the subject invention effective to substantially reduce the likelihood of the non-infected medical practitioner's becoming infected with HIV-1 by virtue of contact with the patient's bodily fluid during the medical procedure.

As used herein, a bodily fluid is any fluid which is present in the human body and is capable of containing infectious HIV-1 in an HIV-1-infected patient. Bodily fluids include, but are not limited to, saliva, cerebrospinal fluid, tears, vaginal secretions, urine, alveolar fluid, synovial fluid and pleural fluid.

Another embodiment of this invention is a method of substantially reducing the likelihood of a non-HIV-1-infected newborn infant's becoming infected with HIV-1 prior to or during birth from an HIV-1-infected mother, which comprises administering to the mother prior to birth an amount of the composition of the subject invention effective to substantially reduce the likelihood of the non-HIV-1-infected newborn infant's becoming infected with HIV-1 by virtue of contact with the patient's bodily fluid.

In order to facilitate an understanding of the Experimental Details section which follows, certain frequently occurring methods and/or terms are best described in Maniatis et al. (23).

This invention will be better understood by reference to the Experimental Details which follow, but those skilled in the art will readily appreciate that the specific experiments detailed are only illustrative of the invention as described more fully in the claims which follow thereafter.

Experimental Details

Nomenclature

As used herein, V3$^{(-)}$ indicates a V3 loop deletion from HIV-1 gp120 envelope glycoprotein. As used herein, CD4$^{(-)}$ indicates a point mutation in the C4 domain of HIV-1 gp120 envelope glycoprotein which mutation inhibits CD4 binding to the mutant HIV-1 gp120 envelope glycoprotein. The structure of HIV-1 gp120 envelope glycoprotein is shown in Figure 1.

Materials and Methods

1. Construction of PPI4-tPA-gp120$_{LAI}$ expression vector. An expression vector was constructed that consisted of the cytomegalovirus major immediate early (CMV MIE) promoter/enhancer linked to the HIV-1$_{LAI}$ env gene, which gene had its signal sequence replaced by the tPA signal sequence. The CMV MIE promoter/enhancer sequences were derived from pSVCC1 (24) consisting of 1580 base pairs of contiguous DNA that is immediately 5' to the initiator ATG. In sequential order, the functional domains of the CMV promoter are: the promoter/enhancer region; a transcriptional initiator site; exon A (a non-coding exon); intron A; and 17 nucleotides of exon B (non-coding sequences). The viral promoter sequences were ligated to a gene construct consisting of the nucleotide sequences encoding amino acids -35 to -1 of human tPA (25) fused in-frame to HIV-1$_{LAI}$ env amino acids 31 through 515, ending with a TGA stop codon. The construction was performed in two parts. The majority of the CMV promoter could be isolated as a 1560 bp Hinc II/Pst I fragment which was ligated to a Pst I/Not I 1590 bp DNA fragment that contained the remainder of the CMV promoter, the initiator ATG, the tPA signal sequence and the mature HIV-1$_{LAI}$ env protein coding sequence.

The latter fragment was assembled using the polymerase chain reaction as follows. Primer 1 (GATCCTGCAGTCACCGTCCTTGACA-CGATGGATGCAATGAAGAGA) and primer 2 (AAGTCTTCTCCTCGGTCTTGT-CTTTTTAACACCCAG) were used to amplify the nucleic acid sequences encoding the tPA signal sequence amino acids -35 to -1 from plasmid pMAM neo-s (Clonetech), thus producing a 150 bp fragment. A second 1440 bp DNA fragment was amplified using primer 3 (TTCAGAAGAGGAGCCAGAACAGAAAAATTGTGGGTC), primer 4 (GGAAAAAAGCGGCCGCTCATTTTTCTCTCTGCACCACTC), and pENV (26) as a template. The PCR fragments were pooled, desalted, and excess primer removed by ultrafiltration through a centricon-100 unit (Amicon). An aliquot of the pooled material was then subjected to a second round of amplification in the presence of primers 1 and 4 to produce a 1590 bp fragment, which was then digested with Pst I and Not I. The CMV promoter fragment and the HIV-1_{LAI} <u>env</u> fragment were then ligated together, and the entire transcription unit subcloned into PPI4, which is a eukaryotic shuttle vector that contains an ampicillin resistance gene, an SV40 origin of replication and a DHFR gene whose transcription is driven by the ß-globin promoter. The final construct, PPI4-tPA-$gp120_{LAI}$, is shown in Figure 2.

The expression vector is then used as the prototype vector for the expression of gp120 proteins that are derived from other HIV-1 strains or mutated as described in the methods section. The vector was constructed so that unique Nar I and Not I sites flank the gp120 sequence, thus facilitating the removal of the gp120 gene cassette and the subsequent insertion of other gene cassettes (Figure 2).

2. <u>Expression of HIV-1_{LAI} gp120 in mammalian cells</u>.

a. <u>Transient expression</u>.

CosM5 cells grown in DMEM containing 10% fetal calf serum

were split to 75% confluence. On the following day, the cells were transfected for 16-20 hours with 10 micrograms of CsCl-purified PPI4-tPA-$gp120_{LAI}$ DNA by the standard $CaPO_4$ (5) precipitation technique. After transfection, fresh medium was added to the cells. Analysis of the products synthesized 96-120 hours post-transfection was performed by radiolabelling the transfectants with ^{35}S-cysteine for 12-18 hours, followed by precipitation of media using a CD4-immunoglobulin-Protein A-Sepharose complex, followed by SDS-PAGE under reducing conditions (Figure 4).

b. Stable expression.

$Dhfr^-$ Chinese hamster ovary cells (CHO) were transfected with 20 micrograms of CsCl-purified DNA. Approximately 3-5 days post-transfection, cells were placed in selective medium (nucleoside-free alpha MEM containing 10% dialyzed fetal calf serum). Approximately 10-15 days post-selection, individual cell clones were picked. Media was analyzed for gp120 expression by radiolabelling the cells with ^{35}S-cysteine for 12-18 hours, followed by precipitation of media using a CD4-immunoglobulin-Protein A-Sepharose complex, followed in turn by SDS-PAGE under reducing conditions (Figure 6). The levels of gp120 in the media of these clones were also quantitated (Figure 5) by ELISA performed as follows. The method involves coating 96-well plates overnight with sheep polyclonal IgG against the highly conserved C-terminus of gp120 (D7234, Aalto Bioreagents). After washing, dilutions of a standard gp120 preparation in cell growth medium, or supernatant from the stably-transfected cells, were incubated for 1 hour. The plates were washed again, and incubated for one hour with a horseradish peroxidase-conjugated anti-gp120 monoclonal antibody (9204, DuPont). Following a final wash, the peroxidase substrate OPD (DuPont) was added and the amount

of gp120 determined by comparing absorbance of unknowns with a standard curve. Standards were prepared from purified gp120 made in CHO cells, a small quantity of which was obtained from Celltech Ltd. Clones expressing the highest levels were subjected to successive rounds of amplification of the newly introduced DNA sequences in increasing concentrations of methotrexate. Stable CHO cell lines were thus generated which secrete at least 1 microgram/milliliter of HIV-1_{LAI} gp120.

3. Construction of PPI4-tPA-$gp120_{JR-FL}$.

a. The HIV-1_{LAI} gp120 env nucleotide sequence in PPI4-tPA-$gp120_{LAI}$ was replaced by the nucleotide sequence encoding the mature $gp120_{JR-FL}$ protein. Using the polymerase chain reaction, the JR-FL sequences were amplified from pUC112-1 (27) using primer 5 (GATCGGCGCCAGAGTAGAAAAGTTGTGGGTCAC) and primer 4. The PCR fragment was digested with the restriction endonucleases Nar I and Not I, and the fragment subcloned in between the Nar I and Not I sites in PPI4-tPA-$gp120_{LAI}$ to generate PPI4-tPA-$gp120_{JR-FL}$ (Figure 7).

b. Transient expression.

CosM5 cells grown in DMEM containing 10% fetal calf serum were split to 75% confluence. On the following day, the cells were transfected for 16-20 hours with 10 micrograms of CsCl-purified PPI4-tPA-$gp120_{JR-FL}$ DNA by the standard $CaPO_4$ (5) precipitation technique. After transfection, fresh medium was added to the cells. Analysis of the products synthesized 96-120 hours post-transfection was performed by radiolabelling the transfectants with ^{35}S-cysteine for 12-18 hours, followed by precipitation of media using a CD4-immunoglobulin-Protein A-Sepharose complex, followed by SDS-PAGE under reducing conditions (Figure 4).

4. Construction of PPI4-tPA-gp120$_{LAI}$-V3$^{(-)}$.

The V3 loop in tPA-gp120$_{LAI}$ consists of amino acids Cys$_{306}$ through Cys$_{333}$. In the V3$^{(-)}$ mutant, the amino acids in between these cysteines are replaced by the pentapeptide sequence Thr-Gly-Ala-Gly-His. Using the Transformer Site-Directed Mutagenesis Kit (Clonetech), the V3 loop sequence in PPI4-tPA-gp120$_{LAI}$ is altered using the mutagenic primer 6 (CTGTAGAAATTAATTGTACAGGTGCTGGACATTGTAACATTAGTAGAGC) and primer 7 (CTCGAGCATGCATTCGAAGCTCGCTGATC) as a selection primer. Primer 7 changes a unique Xba I site in the backbone of the parent PPI4 plasmid into a unique BstB I site. Briefly, the mutagenesis method requires incubating of the parent plasmid with the mutagenic primer and the selection primer, denaturing at 100°C for 3 minutes and then chilling on ice. In the presence of buffered deoxynucleotide triphosphates and T4 DNA polymerase, the primers are allowed to initiate the polymerization of one strand of plasmid DNA. T4 DNA ligase is used to seal the newly synthesized DNA strand to form a covalently closed circle. Hybrid plasmids are then transformed into a MutS strain of E. coli that is deficient in mismatch repair. After allowing for the growth of transformed cells, DNA is purified from the cells and digested with the selection restriction endonuclease, in this case Xba I. Parental plasmids are cleaved by Xba I while the mutant plasmid remains resistant to cleavage by virtue of the Xba I to BstB I conversion. Digested DNA is then used to transform E. coli, and colonies harboring the mutant plasmid are picked. Multiple mutagenic primers can be used in a single round of mutagenesis. The amino acid sequence of the modified protein is shown in Figure 8.

5. Construction of PPI4-tPA-gp120$_{JR-FL}$-V3$^{(-)}$.

The V3 loop in tPA-gp120$_{JR-FL}$ consists of amino acids Cys$_{293}$

through Cys_{327}. In the $V3^{(-)}$ mutant, the amino acids in between these cysteines are replaced by the pentapeptide sequence Thr-Gly-Ala-Gly-His. Using the Transformer Site-Directed Mutagenesis Kit (Clonetech), the V3 loop sequence in PPI4-tPA-$gp120_{JR-FL}$ is altered using the mutagenic primer 6 (CTGTAGAAATTAATTGTACAGGTGCTGGACATTGTAACATTAGTAGAGC) and primer 7 as a selection primer. The amino acid sequence of the modified protein is shown in Figure 9.

6. Construction of PPI4-tPA-$gp120_{LAI}$-$CD4^{(-)}$.

Using the Transformer Site-Directed Mutagenesis Kit (Clonetech), the selection primer 7, and the mutagenic primer 8 (CAATTTATAAACATGGTGCAGGAAGTAGG), Trp_{437} of tPA-$gp120_{LAI}$, which is in an equivalent position to the tryptophan residue in the HXBc2 strain of HIV-1, is mutated to a Val in the expression vector PPI4-tPA-$gp120_{LAI}$ to generate PPI4-tPA-$gp120_{LAI}$-$CD4^{(-)}$. The sequence for $gp120_{LAI}$-$CD4^{(-)}$ is shown in Figure 12.

7. Construction of PPI4-tPA-$gp120_{JR-FL}$-$CD4^{(-)}$.

In a fashion similar to that described above, Trp_{424} of tPA-$gp120_{JR-FL}$ is mutated to a Val in the expression vector PPI4-tPA-$gp120_{JR-FL}$ using the selection primer 7 and the mutagenic primer 9 (CAAATTATAAACATGGTGCAGGAAGTAGG) to generate PPI4-tPA-$gp120_{JR-FL}$-$CD4^{(-)}$. The sequence for $gp120_{JR-FL}$-$CD4^{(-)}$ is shown in Figure 13.

8. Construction of PPI4-tPA-$gp120_{LAI}$-$V3^{(-)}$-$CD4^{(-)}$.

The tPA-$gp120_{LAI}$ double mutant, $V3^{(-)}$-$CD4^{(-)}$, is constructed by including the mutagenic primers 6 and 8, and the selection primer 7 simultaneously in the reaction tube with PPI4-tPA-$gp120_{LAI}$ as the DNA template. The final construct is named PPI4-tPA-$gp120_{LAI}$-$V3^{(-)}$-$CD4^{(-)}$, and its sequence is shown in figure 10.

9. Construction of PPI4-tPA-$gp120_{JR-FL}$-$V3^{(-)}$-$CD4^{(-)}$.

The tPA-$gp120_{JR-FL}$ double mutant, $V3^{(-)}$-$CD4^{(-)}$, is constructed by including the mutagenic primers 6 and 9, and the selection primer 7 simultaneously in the reaction tube with PPI4-tPA-$gp120_{JR-FL}$ as the DNA template. The final construct is named PPI4-tPA-$gp120_{JR-FL}$-$V3^{(-)}$-$CD4^{(-)}$, and its sequence is shown in figure 11.

10. Expression of mutant HIV-1 gp120 in mammalian cells.

a. Transient expression.

CosM5 cells grown in DMEM containing 10% fetal calf serum are split to 75% confluence. On the next day, the cells are transfected for 16-20 hours with 10 micrograms of CsCl-purified mutant HIV-1 DNA by the standard $CaPO_4$ (5) precipitation technique. After transfection, fresh medium is added to the cells. Analysis of the products synthesized 96-120 hours post-transfection is performed by radiolabelling the transfectants with ^{35}S-cysteine for 12-18 hours, followed by precipitation of media using a sheep polyclonal IgG against the highly conserved C-terminus of gp120.

b. Stable expression.

Dhfr$^-$ Chinese hamster ovary cells (CHO) are transfected with 20 micrograms of CsCl-purified DNA encoding the native or mutant HIV-1 gp120 glycoproteins. Approximately 3-5 days post-transfection, cells are placed in selective medium (nucleoside-free alpha MEM containing 10% dialyzed fetal calf serum). Approximately 10-15 days post-selection, individual cell clones are picked. Media is analyzed for gp120 expression by radiolabelling the cells with ^{35}S-cysteine for 12-18 hours, followed by quantitative immunoprecipitation of media using a sheep polyclonal IgG against the highly conserved C-terminus of gp120, followed

in turn by SDS-PAGE under reducing conditions. Alternatively, one can quantitate the level of gp120 by ELISA performed as follows. The method involves coating 96-well plates overnight with sheep polyclonal IgG against the highly conserved C-terminus of gp120 (D7234, Aalto Bioreagents). After washing, dilutions of a standard gp120 preparation in cell growth medium, or supernatant from the stably-transfected cells, are incubated for 1 hour. The plates are washed again, and incubated for one hour with a human MoAb (F105, AIDS Research & Reference Reagent Program, No. 857). The plates are washed again, and incubated again for 1 hour with a horseradish-peroxidase-conjugated goat anti-human IgG (Cappel). Following a final wash, the peroxidase substrate OPD (DuPont) is added and the amount of gp120 determined by comparing absorbance of unknowns with a standard curve. Standards are prepared from purified gp120 made in CHO cells, a small quantity of which is obtained from Celltech Ltd. Clones expressing the highest levels are subjected to successive rounds of amplification of the newly introduced DNA sequences in increasing concentrations of methotrexate. Stable CHO cell lines are thus generated which secrete at least 1 microgram/milliliter of mutant HIV-1 gp120.

11. <u>Purification of HIV-1 gp120 proteins</u>.

A one-step immunoaffinity procedure is used to purify the recombinant gp120 molecules described. Briefly, culture supernatant is collected and clarified by centrifugation. An immunoaffinity column consisting of a matrix coupled to a sheep polyclonal anti-gp120 IgG (D7234, Aalto Bioreagents) directed against the highly conserved C-terminal end (APTKAKRRVVQREKR) of gp120 is used to specifically adsorb gp120 from the cell culture media. This antisera recognizes native gp120, the V3 loop deletion mutants, and the $CD4^{(-)}$

mutants since the C-terminal ends of these molecules remain unaltered. The bound gp120 is then eluted with 2M $MgCl_2$, concentrated by Amicon filtration, and dialyzed into 10 mM HEPES, pH 7.0. The purity of the proteins is determined by SDS-PAGE and silver staining.

12. <u>Characterization of recombinant HIV-1 gp120 proteins</u>. The purified glycoproteins are subjected to extensive biochemical and immunologic characterization. The integrity of the proteins is monitored by SDS-PAGE and silver staining under reducing and non-reducing conditions. The glycoproteins are deglycosylated by treatment with the enzyme N-glycosidase F which cleaves N-linked oligosaccharides, and are assayed by SDS-PAGE and silver staining to monitor molecular weight shifts. The purified glycoproteins are also tested for reactivity with several well characterized anti-gp120 monoclonal antibodies that recognize both linear and discontinuous epitopes. The binding affinity to sCD4 is estimated using an ELISA assay.

The purified proteins HIV-1 $gp120_{LAI}$, $gp120_{LAI}$-$V3^{(-)}$, $gp120_{LAI}$-$V3^{(-)}$-$CD4^{(-)}$, $gp120_{JR-FL}$, $gp120_{JR-FL}$-$V3^{(-)}$, and $gp120_{JR-FL}$-$V3^{(-)}$-$CD4^{(-)}$, were tested for their ability to bind cell surface human CD4. DG44 #3 cells, a recombinant cell line designed to express human CD4 on the membrane surface, were grown in T flasks and trypsinized. 5 X 10^5 cells/experiment were aliquoted into FACS buffer (PBS + 2% BSA and 0.1% NaN_3), washed several times in the same buffer, and then incubated with 100 ul of a solution of purified gp120 protein at 5ug/ml in FACS buffer at 37°C for 2 hr. The cells were washed in FACS buffer, and then incubated in 100 ul solution containing 5ug/ml sheep polyclonal IgG against the highly conserved C-terminus of gp120 in FACS buffer at 37°C for 2 hr. The cells were washed in FACS buffer then incubated in 100 ul

solution containing FITC-labeled rabbit anti-sheep IgG polyclonal antibody at 37°C for 2 hr. The cells were washed with FACS buffer and then resuspended in 500 ul FACS buffer. The cells were then analyzed on a Becton Dickinson FACScan according to the manufacturer's instructions. As a control for expression of CD4 on the DG44 #3 cells, FITC-labeled OKT4A (Becton Dickinson) was used.

13. <u>A protocol for inoculation of animals with the mutant HIV-1 gp120 envelope glycoproteins</u>.

Alum is used as an adjuvant during the inoculation series. The inoculum is prepared by dissolving the mutant HIV-1 gp120 envelope glycoprotein antigen in physiologic saline at a final antigen concentration of 100 ug/ml. Preformed alum (aluminum hydroxide gel) is added to the solution to a final level of 500 ug/ml aluminum. The antigen is allowed to adsorb onto the alum gel for two hours at room temperature. Following adsorption, the gel with the antigen is washed twice with physiologic saline and resuspended in the saline to a protein concentration of 100 ug/ml.

Monkeys and/or Guinea Pigs are individually inoculated with four 100 ug doses of the mutant HIV-1 gp120 envelope glycoprotein antigen adsorbed onto alum. Each dose is injected intramuscularly. The doses are delivered one or five months apart (week 0, 4, 8 and 28). the animals are bled at intervals of two or four weeks. Serum samples are prepared from each bleed to assay for the development of specific antibodies as described in the subsequent sections.

14. <u>Analysis of sera for anti-mutant HIV-1 gp120 envelope glycoprotein IgG antibodies</u>.

Each serum sample is analyzed by ELISA. Polystyrene microtiter plates are coated with 0.5 ug per well of pure mutant HIV-1 gp120 envelope glycoprotein in phosphate-

buffered physiological saline (PBS) at 4°C. Each well is then washed with PBS containing 0.5% TWEEN-20 (PBS-TW). Test serum, diluted serially in PBS-TW, is added to the mutant HIV-1 gp120 envelope glycoprotein-containing wells and allowed to react with the adsorbed mutant HIV-1 gp120 envelope glycoprotein for one hour at 37°C. The wells are then washed extensively in PBS-TW. Each well then receives 0.1% p-nitrophenyl phosphate in 10% diethanolamine, pH 9.8, containing 0.5 mM $MgCl_2.6H_2O$. The ensuing reaction is allowed to proceed at room temperature for 30 minutes, at which time it is terminated by the addition of 3.0 N NaOH.

The greater the interaction of antibodies in the test serum with the mutant HIV-1 gp120 envelope glycoprotein, the greater is the amount of alkaline phosphatase bound onto the well. The phosphatase enzyme mediates the breakdown of p-nitrophenyl phosphate into a molecular substance which absorbs light at a wavelength of 405 nm. Hence, there exists a direct relationship between the absorbance at 405 nm of light at the end of the ELISA reaction and the amount of mutant HIV-1 gp120 envelope glycoprotein-bound antibody. All animals inoculated with mutant HIV-1 gp120 envelope glycoprotein whose serum reacts specifically with the mutant HIV-1 gp120 envelope glycoprotein in the ELISA have a positive antibody response against mutant HIV-1 gp120 envelope glycoprotein.

15. <u>Analysis of sera for activity which specifically neutralizes HIV-1 infectivity</u>.

Virus-neutralizing activity is determined with an assay based on the use of multiplicity curves in which the ratio of infectious virus surviving antibody treatment (V_n) is compared to infectious virus in uninhibited cultures (V_o) at various dilutions of antisera. The neutralization titer of

the sera is then interpolated as that sera dilution which yields one log reduction in infectious titer (i.e., V_n/V_o = 0.1). Briefly, 4-fold dilutions of virus (laboratory-adapted and primary isolates) are prepared to yield infectious doses of 0.1 to 100 $TCID_{50}$ (Tissue Culture Infection Dose) in 20 ul. Serial 3-fold dilutions of sera are also prepared and 20 ul of each serum dilution are incubated with each dilution of virus in duplicate for 60 minutes at room temperature in a 96-well microtiter plate. 20 ul of AA5 cells (PHA stimulated PBMCs for primary HIV-1 isolates) are then added to the serum/virus mixtures. Cells are cultured for 7 days by the addition of fresh medium every other day. On the seventh day, supernatant from each well is removed and tested for the presence of reverse transcriptase (RT). Infection in each well is then scored as either positive or negative based on the RT counts, and the infectious dose of virus in each treatment group is calculated using the Reed and Muench (28) formula. The neutralization titers represent the reciprocal serum dilution required to reduced infectious dose of virus by one log. The above culture time is for the prototypic HIV-1_{LAI} isolate tested on the AA5 cell line. In the case of primary isolates, the termination date is usually 11-14 days. Culture conditions for PBMCs is not as demanding since doubling time is restricted. In the case of PBMCs, one day PHA stimulations are used at a final concentration of 1.5 X 10^6/ml on day 0. Half that number of fresh PBMCs are then added again on days 4 and 8. This multiple addition of PBMCs is meant to amplify virus output upon successful infection so that the readout RT signal is strong. Again, the final readout titer for the primary isolate/PBMC is the reciprocal serum dilution which reduces infectious titer by one log.

16. Passive hyperimmune therapy.

Non-HIV-1-infected humans are immunized with the mutant HIV-1 gp120 envelope glycoprotein antigens according to a protocol similar to that described above in section 12. For passive hyperimmune therapy in HIV-1-infected individuals, blood plasma is taken from mutant HIV-1 gp120 envelope glycoprotein immunized, non-HIV-1-infected human donors whose plasma has high levels of neutralizing antibodies. The plasma is pooled from several donors, purified to remove nonimmunoglobulin proteins and is then sterilized to kill any other viruses or pathogens. The treated plasma is then injected into individuals infected with HIV-1, with repeated injections every week, every two weeks, or every month.

Results

Eukaryotic expression vectors designed to express high levels of HIV-1_{LAI} gp120 and HIV-1_{JR-FL} gp120 were constructed. The CMV MIE promoter/enhancer was used to drive the transcription of a gene fusion consisting of the human tPA signal sequence fused to mature gp120 (Figures 2 and 7). The complete sequence of the transcription unit from the Hinc II site of the CMV promoter/enhancer to the Not I site just 3' from the stop codon in gp120 is shown in figure 3. This vector was used to transfect COSM5 cells in a transient assay. The transfected cells were labeled with ^{35}S-cysteine and the media immunoprecipitated with a CD4-immunoglobulin-Protein A-Sepharose complex. The precipitated products were analyzed using a reducing 10% SDS-PAGE gel and autoradiography (Figure 4). A 120 kD band was detected when PPI4-tPA-$gp120_{LAI}$ was used to transfect COS cells (lane 3). A band migrating with a slightly lower molecular mass was detected when PPI4-tPA-$gp120_{JR-FL}$ was used to transfect COS cells (lane 4). No radiolabeled products were detected in the mock infected cells. Using a sheep polyclonal antibody directed against the highly conserved C-terminal end of HIV-1 gp120 in an ELISA assay, the level of expression of HIV-1 gp120 was determined to be 2350 ng/ml.

The PPI4-tPA-$gp120_{LAI}$ vector was then used to stably transfect the dhfr$^-$ CHO cell line DXB11. Two days post-transfection, the cells were plated at low density in nucleoside-free medium. Eight days post-transfection, surviving clones were isolated and expanded. Individual primary transfectants were tested for gp120 expression using the ELISA method described in the methods section. Several primary CHO transfectants expressed significant quantities (10-120 ng/ml) of gp120 (Figure 5). Three of the highest

expressing clones were then subjected to increasing concentrations of methotrexate in order to amplify, in tandem, the copy number of the dhfr and gp120 genes. Cell lines were established that express high levels of gp120 with rates of secretion greater than 1 mg/liter. These were then used to purify gp120 to homogeneity.

Six CHO cell lines were established, using the procedures described in the methods sections, that express high levels of the following proteins: HIV-1 $gp120_{LAI}$, $gp120_{LAI}$-$V3^{(-)}$, $gp120_{LAI}$-$V3^{(-)}$-$CD4^{(-)}$, $gp120_{JR-FL}$, $gp120_{JR-FL}$-$V3^{(-)}$, and $gp120_{JR-FL}$-$V3^{(-)}$-$CD4^{(-)}$. Metabolic labeling of these cells with ^{35}S-cysteine followed by immunoprecipitation with the human monoclonal antibody F105 and analyzed by SDS-PAGE and autoradiography showed the presence of the gp120 proteins in the culture supernatant (Figure 14). From these cell lines the gp120 proteins were purified to homogeneity. Analysis by SDS-PAGE followed by silver-staining showed the purity of these proteins to be greater than 90% (Figure 15).

It was shown by FACScan analysis that the two CD4 binding mutants HIV-1$gp120_{LAI}$-$V3^{(-)}$-$CD4^{(-)}$ and HIV-1 $gp120_{JR-FL}$-$V3^{(-)}$-$CD4^{(-)}$ had no appreciable binding to recombinant cell lines designed to express high levels of human CD4 on their membrane surface (Figure 16, panel 4 and data not shown, respectively).

Discussion

The advantage of using the mutant HIV-1 gp120 envelope glycoproteins as immunogens is that these proteins will not elicit an immune response against the V3 loop, a highly immunodominant epitope on gp120. This is significant because the V3 loop may skew the humoral immune response away from discontinuous epitopes in the CD4-binding site. Mutant HIV-1 gp120 envelope glycoproteins having partial and total V3 loop deletions have been made (30). Deletion of the V3 loop therefore exposes the CD4-binding site to the immune system, allowing the immune system to mount a response against this critical region (18). Another advantage of using the mutant HIV-1 gp120 envelope glycoprotein as an immunogen is that it has significantly reduced affinity for cell surface CD4. An efficient humoral immune response depends on the binding of antigen to B cell surface immunoglobulin. The presence of the high-affinity CD4 receptor on large numbers of cells in the body may significantly diminish the ability of native gp120 to induce an effective humoral immune response. The rationale of mutating gp120 at the CD4 binding site is to redirect the mutant HIV-1 gp120 envelope glycoprotein away from cell surface CD4 toward immunoglobulin-bearing B cells, thereby allowing the immune system to mount a response against, inter alia, the CD4-binding site.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Progenics Pharmaceuticals, Inc.

(ii) TITLE OF INVENTION: HIV-1 VACCINES, ANTIBODY COMPOSITIONS RELATED THERETO, AND THERAPEUTIC AND PROPHYLACTIC USES THEREOF

(iii) NUMBER OF SEQUENCES: 29

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.24

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/037,816
(B) FILING DATE: 26-MAR-1993

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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Xaa Xaa Ala Xaa Tyr Xaa Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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(i) SEQUENCE CHARACTERISTICS:
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(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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(2) INFORMATION FOR SEQ ID NO:7:

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(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

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(2) INFORMATION FOR SEQ ID NO:9:

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(A) LENGTH: 49 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

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(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

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(2) INFORMATION FOR SEQ ID NO:11:

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(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

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(2) INFORMATION FOR SEQ ID NO:12:

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(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

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(2) INFORMATION FOR SEQ ID NO:13:

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(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1555..3115
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

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                                                            Met
                                                              1

GAT GCA ATG AAG AGA GGG CTC TGC TGT GTG CTG CTG CTG TGT GGA GCA      1605
Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly Ala
                5                  10                  15

GTC TTC GTT TCG CCC AGC CAG GAA ATC CAT GCC CGA TTC AGA AGA GGC      1653
Val Phe Val Ser Pro Ser Gln Glu Ile His Ala Arg Phe Arg Arg Gly
            20                  25                  30

GCC AGA ACA GAA AAA TTG TGG GTC ACA GTC TAT TAT GGG GTA CCT GTG      1701
Ala Arg Thr Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val
        35                  40                  45

TGG AAG GAA GCA ACC ACC ACT CTA TTT TGT GCA TCA GAT GCT AAA GCA      1749
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Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala
 50                 55                  60                  65

TAT GAT ACA GAG GTA CAT AAT GTT TGG GCC ACA CAT GCC TGT GTA CCC      1797
Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val Pro
                 70                  75                  80

ACA GAC CCC AAC CCA CAA GAA GTA GTA TTG GTA AAT GTG ACA GAA AAT      1845
Thr Asp Pro Asn Pro Gln Glu Val Val Leu Val Asn Val Thr Glu Asn
             85                  90                  95

TTT AAC ATG TGG AAA AAT GAC ATG GTA GAA CAG ATG CAT GAG GAT ATA      1893
Phe Asn Met Trp Lys Asn Asp Met Val Glu Gln Met His Glu Asp Ile
        100                 105                 110

ATC AGT TTA TGG GAT CAA AGC CTA AAG CCA TGT GTA AAA TTA ACC CCA      1941
Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr Pro
    115                 120                 125

CTC TGT GTT AGT TTA AAG TGC ACT GAT TTG GGG AAT GCT ACT AAT ACC      1989
Leu Cys Val Ser Leu Lys Cys Thr Asp Leu Gly Asn Ala Thr Asn Thr
130                 135                 140                 145

AAT AGT AGT AAT ACC AAT AGT AGT AGC GGG GAA ATG ATG ATG GAG AAA      2037
Asn Ser Ser Asn Thr Asn Ser Ser Ser Gly Glu Met Met Met Glu Lys
                150                 155                 160

GGA GAG ATA AAA AAC TGC TCT TTC AAT ATC AGC ACA AGC ATA AGA GGT      2085
Gly Glu Ile Lys Asn Cys Ser Phe Asn Ile Ser Thr Ser Ile Arg Gly
            165                 170                 175

AAG GTG CAG AAA GAA TAT GCA TTT TTT TAT AAA CTT GAT ATA ATA CCA      2133
Lys Val Gln Lys Glu Tyr Ala Phe Phe Tyr Lys Leu Asp Ile Ile Pro
        180                 185                 190

ATA GAT AAT GAT ACT ACC AGC TAT ACG TTG ACA AGT TGT AAC ACC TCA      2181
Ile Asp Asn Asp Thr Thr Ser Tyr Thr Leu Thr Ser Cys Asn Thr Ser
    195                 200                 205

GTC ATT ACA CAG GCC TGT CCA AAG GTA TCC TTT GAG CCA ATT CCC ATA      2229
Val Ile Thr Gln Ala Cys Pro Lys Val Ser Phe Glu Pro Ile Pro Ile
210                 215                 220                 225

CAT TAT TGT GCC CCG GCT GGT TTT GCG ATT CTA AAA TGT AAT AAT AAG      2277
His Tyr Cys Ala Pro Ala Gly Phe Ala Ile Leu Lys Cys Asn Asn Lys
                230                 235                 240

ACG TTC AAT GGA ACA GGA CCA TGT ACA AAT GTC AGC ACA GTA CAA TGT      2325
Thr Phe Asn Gly Thr Gly Pro Cys Thr Asn Val Ser Thr Val Gln Cys
            245                 250                 255

ACA CAT GGA ATT AGG CCA GTA GTA TCA ACT CAA CTG CTG TTG AAT GGC      2373
Thr His Gly Ile Arg Pro Val Val Ser Thr Gln Leu Leu Leu Asn Gly
        260                 265                 270

AGT CTA GCA GAA GAA GAG GTA GTA ATT AGA TCT GCC AAT TTC ACA GAC      2421
Ser Leu Ala Glu Glu Glu Val Val Ile Arg Ser Ala Asn Phe Thr Asp
    275                 280                 285

AAT GCT AAA ACC ATA ATA GTA CAG CTG AAC CAA TCT GTA GAA ATT AAT      2469
Asn Ala Lys Thr Ile Ile Val Gln Leu Asn Gln Ser Val Glu Ile Asn
290                 295                 300                 305

TGT ACA AGA CCC AAC AAC AAT ACA AGA AAA AGT ATC CGT ATC CAG AGG      2517
Cys Thr Arg Pro Asn Asn Asn Thr Arg Lys Ser Ile Arg Ile Gln Arg
                310                 315                 320

GGA CCA GGG AGA GCA TTT GTT ACA ATA GGA AAA ATA GGA AAT ATG AGA      2565
Gly Pro Gly Arg Ala Phe Val Thr Ile Gly Lys Ile Gly Asn Met Arg
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325 330 335

CAA GCA CAT TGT AAC ATT AGT AGA GCA AAA TGG AAT GCC ACT TTA AAA 2613
Gln Ala His Cys Asn Ile Ser Arg Ala Lys Trp Asn Ala Thr Leu Lys
340 345 350

CAG ATA GCT AGC AAA TTA AGA GAA CAA TTT GGA AAT AAT AAA ACA ATA 2661
Gln Ile Ala Ser Lys Leu Arg Glu Gln Phe Gly Asn Asn Lys Thr Ile
355 360 365

ATC TTT AAG CAA TCC TCA GGA GGG GAC CCA GAA ATT GTA ACG CAC AGT 2709
Ile Phe Lys Gln Ser Ser Gly Gly Asp Pro Glu Ile Val Thr His Ser
370 375 380 385

TTT AAT TGT GGA GGG GAA TTT TTC TAC TGT AAT TCA ACA CAA CTG TTT 2757
Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys Asn Ser Thr Gln Leu Phe
390 395 400

AAT AGT ACT TGG TTT AAT AGT ACT TGG AGT ACT GAA GGG TCA AAT AAC 2805
Asn Ser Thr Trp Phe Asn Ser Thr Trp Ser Thr Glu Gly Ser Asn Asn
405 410 415

ACT GAA GGA AGT GAC ACA ATC ACA CTC CCA TGC AGA ATA AAA CAA TTT 2853
Thr Glu Gly Ser Asp Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln Phe
420 425 430

ATA AAC ATG TGG CAG GAA GTA GGA AAA GCA ATG TAT GCC CCT CCC ATC 2901
Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro Ile
435 440 445

AGC GGA CAA ATT AGA TGT TCA TCA AAT ATT ACA GGG CTG CTA TTA ACA 2949
Ser Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr Gly Leu Leu Leu Thr
450 455 460 465

AGA GAT GGT GGT AAT AAC AAC AAT GGG TCC GAG ATC TTC AGA CCT GGA 2997
Arg Asp Gly Gly Asn Asn Asn Asn Gly Ser Glu Ile Phe Arg Pro Gly
470 475 480

GGA GGA GAT ATG AGG GAC AAT TGG AGA AGT GAA TTA TAT AAA TAT AAA 3045
Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr Lys Tyr Lys
485 490 495

GTA GTA AAA ATT GAA CCA TTA GGA GTA GCA CCC ACC AAG GCA AAG AGA 3093
Val Val Lys Ile Glu Pro Leu Gly Val Ala Pro Thr Lys Ala Lys Arg
500 505 510

AGA GTG GTG CAG AGA GAA AAA T GAGCGGCCGC 3125
Arg Val Val Gln Arg Glu Lys
515 520

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 520 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
1 5 10 15

Ala Val Phe Val Ser Pro Ser Gln Glu Ile His Ala Arg Phe Arg Arg
20 25 30

Gly Ala Arg Thr Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro

35 40 45

Val Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
50 55 60

Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
65 70 75 80

Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Val Asn Val Thr Glu
85 90 95

Asn Phe Asn Met Trp Lys Asn Asp Met Val Glu Gln Met His Glu Asp
100 105 110

Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
115 120 125

Pro Leu Cys Val Ser Leu Lys Cys Thr Asp Leu Gly Asn Ala Thr Asn
130 135 140

Thr Asn Ser Ser Asn Thr Asn Ser Ser Ser Gly Glu Met Met Met Glu
145 150 155 160

Lys Gly Glu Ile Lys Asn Cys Ser Phe Asn Ile Ser Thr Ser Ile Arg
165 170 175

Gly Lys Val Gln Lys Glu Tyr Ala Phe Phe Tyr Lys Leu Asp Ile Ile
180 185 190

Pro Ile Asp Asn Asp Thr Thr Ser Tyr Thr Leu Thr Ser Cys Asn Thr
195 200 205

Ser Val Ile Thr Gln Ala Cys Pro Lys Val Ser Phe Glu Pro Ile Pro
210 215 220

Ile His Tyr Cys Ala Pro Ala Gly Phe Ala Ile Leu Lys Cys Asn Asn
225 230 235 240

Lys Thr Phe Asn Gly Thr Gly Pro Cys Thr Asn Val Ser Thr Val Gln
245 250 255

Cys Thr His Gly Ile Arg Pro Val Val Ser Thr Gln Leu Leu Leu Asn
260 265 270

Gly Ser Leu Ala Glu Glu Glu Val Val Ile Arg Ser Ala Asn Phe Thr
275 280 285

Asp Asn Ala Lys Thr Ile Ile Val Gln Leu Asn Gln Ser Val Glu Ile
290 295 300

Asn Cys Thr Arg Pro Asn Asn Asn Thr Arg Lys Ser Ile Arg Ile Gln
305 310 315 320

Arg Gly Pro Gly Arg Ala Phe Val Thr Ile Gly Lys Ile Gly Asn Met
325 330 335

Arg Gln Ala His Cys Asn Ile Ser Arg Ala Lys Trp Asn Ala Thr Leu
340 345 350

Lys Gln Ile Ala Ser Lys Leu Arg Glu Gln Phe Gly Asn Asn Lys Thr
355 360 365

Ile Ile Phe Lys Gln Ser Ser Gly Gly Asp Pro Glu Ile Val Thr His
370 375 380

Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys Asn Ser Thr Gln Leu
385 390 395 400

Phe Asn Ser Thr Trp Phe Asn Ser Thr Trp Ser Thr Glu Gly Ser Asn

405 410 415

Asn Thr Glu Gly Ser Asp Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln
420 425 430

Phe Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro
435 440 445

Ile Ser Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr Gly Leu Leu Leu
450 455 460

Thr Arg Asp Gly Gly Asn Asn Asn Asn Gly Ser Glu Ile Phe Arg Pro
465 470 475 480

Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr Lys Tyr
485 490 495

Lys Val Val Lys Ile Glu Pro Leu Gly Val Ala Pro Thr Lys Ala Lys
500 505 510

Arg Arg Val Val Gln Arg Glu Lys
515 520

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1532 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1522
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

ATG GAT GCA ATG AAG AGA GGG CTC TGC TGT GTG CTG CTG CTG TGT GGA 48
Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
1 5 10 15

GCA GTC TTC GTT TCG CCC AGC CAG GAA ATC CAT GCC CGA TTC AGA AGA 96
Ala Val Phe Val Ser Pro Ser Gln Glu Ile His Ala Arg Phe Arg Arg
20 25 30

GGC GGC AGA GTA GAA AAG TTG TGG GTC ACA GTC TAT TAT GGG GTA CCT 144
Gly Gly Arg Val Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
35 40 45

GTG TGG AAA GAA GCA ACC ACC ACT CTA TTT TGT GCA TCA GAT GCT AAA 192
Val Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
50 55 60

GCA TAT GAT ACA GAG GTA CAT AAT GTT TGG GCC ACA CAT GCC TGT GTA 240
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
65 70 75 80

CCC ACA GAC CCC AAC CCA CAA GAA GTA GTA TTG GAA AAT GTA ACA GAA 288
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Glu Asn Val Thr Glu
85 90 95

CAT TTT AAC ATG TGG AAA AAT AAC ATG GTA GAA CAG ATG CAG GAG GAT 336
His Phe Asn Met Trp Lys Asn Asn Met Val Glu Gln Met Gln Glu Asp
100 105 110

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ATA ATC AGT TTA TGG GAT CAA AGC CTA AAG CCA TGT GTA AAA TTA ACC     384
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
        115                 120                 125

CCA CTC TGT GTT ACT TTA AAT TGC AAG GAT GTG AAT GCT ACT AAT ACC     432
Pro Leu Cys Val Thr Leu Asn Cys Lys Asp Val Asn Ala Thr Asn Thr
    130                 135                 140

ACT AAT GAT AGC GAG GGA ACG ATG GAG AGA GGA GAA ATA AAA AAC TGC     480
Thr Asn Asp Ser Glu Gly Thr Met Glu Arg Gly Glu Ile Lys Asn Cys
145                 150                 155                 160

TCT TTC AAT ATC ACC ACA AGC ATA AGA GAT GAG GTG CAG AAA GAA TAT     528
Ser Phe Asn Ile Thr Thr Ser Ile Arg Asp Glu Val Gln Lys Glu Tyr
                165                 170                 175

GCT CTT TTT TAT AAA CTT GAT GTA GTA CCA ATA GAT AAT AAT AAT ACC     576
Ala Leu Phe Tyr Lys Leu Asp Val Val Pro Ile Asp Asn Asn Asn Thr
            180                 185                 190

AGC TAT AGG TTG ATA AGT TGT GAC ACC TCA GTC ATT ACA CAG GCC TGT     624
Ser Tyr Arg Leu Ile Ser Cys Asp Thr Ser Val Ile Thr Gln Ala Cys
        195                 200                 205

CCA AAG ATA TCC TTT GAG CCA ATT CCC ATA CAT TAT TGT GCC CCG GCT     672
Pro Lys Ile Ser Phe Glu Pro Ile Pro Ile His Tyr Cys Ala Pro Ala
    210                 215                 220

GGT TTT GCG ATT CTA AAG TGT AAT GAT AAG ACG TTC AAT GGA AAA GGA     720
Gly Phe Ala Ile Leu Lys Cys Asn Asp Lys Thr Phe Asn Gly Lys Gly
225                 230                 235                 240

CCA TGT AAA AAT GTC AGC ACA GTA CAA TGT ACA CAT GGA ATT AGG CCA     768
Pro Cys Lys Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro
                245                 250                 255

GTA GTA TCA ACT CAA CTG CTG CTA AAT GGC AGT CTA GCA GAA GAA GAG     816
Val Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu
            260                 265                 270

GTA GTA ATT AGA TCT GAC AAT TTC ACG AAC AAT GCT AAA ACC ATA ATA     864
Val Val Ile Arg Ser Asp Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile
        275                 280                 285

GTA CAG CTG AAA GAA TCT GTA GAA ATT AAT TGT ACA AGA CCC AAC AAC     912
Val Gln Leu Lys Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn
    290                 295                 300

AAT ACA AGA AAA AGT ATA CAT ATA GGA CCA GGG AGA GCA TTT TAT ACT     960
Asn Thr Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr
305                 310                 315                 320

ACA GGA GAA ATA ATA GGA GAT ATA AGA CAA GCA CAT TGT AAC ATT AGT    1008
Thr Gly Glu Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser
                325                 330                 335

AGA GCA AAA TGG AAT GAC ACT TTA AAA CAG ATA GTT ATA AAA TTA AGA    1056
Arg Ala Lys Trp Asn Asp Thr Leu Lys Gln Ile Val Ile Lys Leu Arg
            340                 345                 350

GAA CAA TTT GAG AAT AAA ACA ATA GTC TTT AAT CAC TCC TCA GGA GGG    1104
Glu Gln Phe Glu Asn Lys Thr Ile Val Phe Asn His Ser Ser Gly Gly
        355                 360                 365

GAC CCA GAA ATT GTA ATG CAC AGT TTT AAT TGT GGA GGA GAA TTT TTC    1152
Asp Pro Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe
    370                 375                 380

TAC TGT AAT TCA ACA CAA CTG TTT AAT AGT ACT TGG AAT AAT AAT ACT    1200
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Tyr Cys Asn Ser Thr Gln Leu Phe Asn Ser Thr Trp Asn Asn Asn Thr
385 390 395 400

GAA GGG TCA AAT AAC ACT GAA GGA AAT ACT ATC ACA CTC CCA TGC AGA 1248
Glu Gly Ser Asn Asn Thr Glu Gly Asn Thr Ile Thr Leu Pro Cys Arg
405 410 415

ATA AAA CAA ATT ATA AAC ATG TGG CAG GAA GTA GGA AAA GCA ATG TAT 1296
Ile Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr
420 425 430

GCC CCT CCC ATC AGA GGA CAA ATT AGA TGT TCA TCA AAT ATT ACA GGG 1344
Ala Pro Pro Ile Arg Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr Gly
435 440 445

CTG CTA TTA ACA AGA GAT GGT GGT ATT AAT GAG AAT GGG ACC GAG ATC 1392
Leu Leu Leu Thr Arg Asp Gly Gly Ile Asn Glu Asn Gly Thr Glu Ile
450 455 460

TTC AGA CCT GGA GGA GGA GAT ATG AGG GAC AAT TGG AGA AGT GAA TTA 1440
Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu
465 470 475 480

TAT AAA TAT AAA GTA GTA AAA ATT GAA CCA TTA GGA GTA GCA CCC ACC 1488
Tyr Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Val Ala Pro Thr
485 490 495

AAG GCA AAG AGA AGA GTG GTG CAA AGA GAA AAA T GAGCGGCCGC 1532
Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys
500 505

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 507 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
1 5 10 15

Ala Val Phe Val Ser Pro Ser Gln Glu Ile His Ala Arg Phe Arg Arg
20 25 30

Gly Gly Arg Val Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
35 40 45

Val Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
50 55 60

Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
65 70 75 80

Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Glu Asn Val Thr Glu
85 90 95

His Phe Asn Met Trp Lys Asn Asn Met Val Glu Gln Met Gln Glu Asp
100 105 110

Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
115 120 125

Pro Leu Cys Val Thr Leu Asn Cys Lys Asp Val Asn Ala Thr Asn Thr
130 135 140

Thr Asn Asp Ser Glu Gly Thr Met Glu Arg Gly Glu Ile Lys Asn Cys
145 150 155 160

Ser Phe Asn Ile Thr Thr Ser Ile Arg Asp Glu Val Gln Lys Glu Tyr
165 170 175

Ala Leu Phe Tyr Lys Leu Asp Val Val Pro Ile Asp Asn Asn Asn Thr
180 185 190

Ser Tyr Arg Leu Ile Ser Cys Asp Thr Ser Val Ile Thr Gln Ala Cys
195 200 205

Pro Lys Ile Ser Phe Glu Pro Ile Pro Ile His Tyr Cys Ala Pro Ala
210 215 220

Gly Phe Ala Ile Leu Lys Cys Asn Asp Lys Thr Phe Asn Gly Lys Gly
225 230 235 240

Pro Cys Lys Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro
245 250 255

Val Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu
260 265 270

Val Val Ile Arg Ser Asp Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile
275 280 285

Val Gln Leu Lys Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn
290 295 300

Asn Thr Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr
305 310 315 320

Thr Gly Glu Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser
325 330 335

Arg Ala Lys Trp Asn Asp Thr Leu Lys Gln Ile Val Ile Lys Leu Arg
340 345 350

Glu Gln Phe Glu Asn Lys Thr Ile Val Phe Asn His Ser Ser Gly Gly
355 360 365

Asp Pro Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe
370 375 380

Tyr Cys Asn Ser Thr Gln Leu Phe Asn Ser Thr Trp Asn Asn Asn Thr
385 390 395 400

Glu Gly Ser Asn Asn Thr Glu Gly Asn Thr Ile Thr Leu Pro Cys Arg
405 410 415

Ile Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr
420 425 430

Ala Pro Pro Ile Arg Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr Gly
435 440 445

Leu Leu Leu Thr Arg Asp Gly Gly Ile Asn Glu Asn Gly Thr Glu Ile
450 455 460

Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu
465 470 475 480

Tyr Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Val Ala Pro Thr
485 490 495

Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys
500 505

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1484 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1474
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

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ATG GAT GCA ATG AAG AGA GGG CTC TGC TGT GTG CTG CTG CTG TGT GGA      48
Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
  1               5                  10                  15

GCA GTC TTC GTT TCG CCC AGC CAG GAA ATC CAT GCC CGA TTC AGA AGA      96
Ala Val Phe Val Ser Pro Ser Gln Glu Ile His Ala Arg Phe Arg Arg
             20                  25                  30

GGC GCC AGA ACA GAA AAA TTG TGG GTC ACA GTC TAT TAT GGG GTA CCT     144
Gly Ala Arg Thr Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
         35                  40                  45

GTG TGG AAG GAA GCA ACC ACC ACT CTA TTT TGT GCA TCA GAT GCT AAA     192
Val Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
     50                  55                  60

GCA TAT GAT ACA GAG GTA CAT AAT GTT TGG GCC ACA CAT GCC TGT GTA     240
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
 65                  70                  75                  80

CCC ACA GAC CCC AAC CCA CAA GAA GTA GTA TTG GTA AAT GTG ACA GAA     288
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Val Asn Val Thr Glu
                 85                  90                  95

AAT TTT AAC ATG TGG AAA AAT GAC ATG GTA GAA CAG ATG CAT GAG GAT     336
Asn Phe Asn Met Trp Lys Asn Asp Met Val Glu Gln Met His Glu Asp
            100                 105                 110

ATA ATC AGT TTA TGG GAT CAA AGC CTA AAG CCA TGT GTA AAA TTA ACC     384
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
        115                 120                 125

CCA CTC TGT GTT AGT TTA AAG TGC ACT GAT TTG GGG AAT GCT ACT AAT     432
Pro Leu Cys Val Ser Leu Lys Cys Thr Asp Leu Gly Asn Ala Thr Asn
    130                 135                 140

ACC AAT AGT AGT AAT ACC AAT AGT AGT AGC GGG GAA ATG ATG ATG GAG     480
Thr Asn Ser Ser Asn Thr Asn Ser Ser Ser Gly Glu Met Met Met Glu
145                 150                 155                 160

AAA GGA GAG ATA AAA AAC TGC TCT TTC AAT ATC AGC ACA AGC ATA AGA     528
Lys Gly Glu Ile Lys Asn Cys Ser Phe Asn Ile Ser Thr Ser Ile Arg
                165                 170                 175

GGT AAG GTG CAG AAA GAA TAT GCA TTT TTT TAT AAA CTT GAT ATA ATA     576
Gly Lys Val Gln Lys Glu Tyr Ala Phe Phe Tyr Lys Leu Asp Ile Ile
            180                 185                 190

CCA ATA GAT AAT GAT ACT ACC AGC TAT ACG TTG ACA AGT TGT AAC ACC     624
Pro Ile Asp Asn Asp Thr Thr Ser Tyr Thr Leu Thr Ser Cys Asn Thr
        195                 200                 205
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TCA GTC ATT ACA CAG GCC TGT CCA AAG GTA TCC TTT GAG CCA ATT CCC 672
Ser Val Ile Thr Gln Ala Cys Pro Lys Val Ser Phe Glu Pro Ile Pro
210 215 220

ATA CAT TAT TGT GCC CCG GCT GGT TTT GCG ATT CTA AAA TGT AAT AAT 720
Ile His Tyr Cys Ala Pro Ala Gly Phe Ala Ile Leu Lys Cys Asn Asn
225 230 235 240

AAG ACG TTC AAT GGA ACA GGA CCA TGT ACA AAT GTC AGC ACA GTA CAA 768
Lys Thr Phe Asn Gly Thr Gly Pro Cys Thr Asn Val Ser Thr Val Gln
245 250 255

TGT ACA CAT GGA ATT AGG CCA GTA GTA TCA ACT CAA CTG CTG TTG AAT 816
Cys Thr His Gly Ile Arg Pro Val Val Ser Thr Gln Leu Leu Leu Asn
260 265 270

GGC AGT CTA GCA GAA GAA GAG GTA GTA ATT AGA TCT GCC AAT TTC ACA 864
Gly Ser Leu Ala Glu Glu Glu Val Val Ile Arg Ser Ala Asn Phe Thr
275 280 285

GAC AAT GCT AAA ACC ATA ATA GTA CAG CTG AAC CAA TCT GTA GAA ATT 912
Asp Asn Ala Lys Thr Ile Ile Val Gln Leu Asn Gln Ser Val Glu Ile
290 295 300

AAT TGT ACA GGT GCT GGA CAT TGT AAC ATT AGT AGA GCA AAA TGG AAT 960
Asn Cys Thr Gly Ala Gly His Cys Asn Ile Ser Arg Ala Lys Trp Asn
305 310 315 320

GCC ACT TTA AAA CAG ATA GCT AGC AAA TTA AGA GAA CAA TTT GGA AAT 1008
Ala Thr Leu Lys Gln Ile Ala Ser Lys Leu Arg Glu Gln Phe Gly Asn
325 330 335

AAT AAA ACA ATA ATC TTT AAG CAA TCC TCA GGA GGG GAC CCA GAA ATT 1056
Asn Lys Thr Ile Ile Phe Lys Gln Ser Ser Gly Gly Asp Pro Glu Ile
340 345 350

GTA ACG CAC AGT TTT AAT TGT GGA GGG GAA TTT TTC TAC TGT AAT TCA 1104
Val Thr His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys Asn Ser
355 360 365

ACA CAA CTG TTT AAT AGT ACT TGG TTT AAT AGT ACT TGG AGT ACT GAA 1152
Thr Gln Leu Phe Asn Ser Thr Trp Phe Asn Ser Thr Trp Ser Thr Glu
370 375 380

GGG TCA AAT AAC ACT GAA GGA AGT GAC ACA ATC ACA CTC CCA TGC AGA 1200
Gly Ser Asn Asn Thr Glu Gly Ser Asp Thr Ile Thr Leu Pro Cys Arg
385 390 395 400

ATA AAA CAA TTT ATA AAC ATG TGG CAG GAA GTA GGA AAA GCA ATG TAT 1248
Ile Lys Gln Phe Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr
405 410 415

GCC CCT CCC ATC AGC GGA CAA ATT AGA TGT TCA TCA AAT ATT ACA GGG 1296
Ala Pro Pro Ile Ser Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr Gly
420 425 430

CTG CTA TTA ACA AGA GAT GGT GGT AAT AAC AAC AAT GGG TCC GAG ATC 1344
Leu Leu Leu Thr Arg Asp Gly Gly Asn Asn Asn Asn Gly Ser Glu Ile
435 440 445

TTC AGA CCT GGA GGA GGA GAT ATG AGG GAC AAT TGG AGA AGT GAA TTA 1392
Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu
450 455 460

TAT AAA TAT AAA GTA GTA AAA ATT GAA CCA TTA GGA GTA GCA CCC ACC 1440
Tyr Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Val Ala Pro Thr
465 470 475 480

AAG GCA AAG AGA AGA GTG GTG CAG AGA GAA AAA T GAGCGGCCGC 1484

Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys
485 490

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 491 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
1 5 10 15

Ala Val Phe Val Ser Pro Ser Gln Glu Ile His Ala Arg Phe Arg Arg
20 25 30

Gly Ala Arg Thr Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
35 40 45

Val Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
50 55 60

Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
65 70 75 80

Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Val Asn Val Thr Glu
85 90 95

Asn Phe Asn Met Trp Lys Asn Asp Met Val Glu Gln Met His Glu Asp
100 105 110

Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
115 120 125

Pro Leu Cys Val Ser Leu Lys Cys Thr Asp Leu Gly Asn Ala Thr Asn
130 135 140

Thr Asn Ser Ser Asn Thr Asn Ser Ser Ser Gly Glu Met Met Met Glu
145 150 155 160

Lys Gly Glu Ile Lys Asn Cys Ser Phe Asn Ile Ser Thr Ser Ile Arg
165 170 175

Gly Lys Val Gln Lys Glu Tyr Ala Phe Phe Tyr Lys Leu Asp Ile Ile
180 185 190

Pro Ile Asp Asn Asp Thr Thr Ser Tyr Thr Leu Thr Ser Cys Asn Thr
195 200 205

Ser Val Ile Thr Gln Ala Cys Pro Lys Val Ser Phe Glu Pro Ile Pro
210 215 220

Ile His Tyr Cys Ala Pro Ala Gly Phe Ala Ile Leu Lys Cys Asn Asn
225 230 235 240

Lys Thr Phe Asn Gly Thr Gly Pro Cys Thr Asn Val Ser Thr Val Gln
245 250 255

Cys Thr His Gly Ile Arg Pro Val Val Ser Thr Gln Leu Leu Leu Asn
260 265 270

Gly Ser Leu Ala Glu Glu Glu Val Val Ile Arg Ser Ala Asn Phe Thr
275 280 285

Asp Asn Ala Lys Thr Ile Ile Val Gln Leu Asn Gln Ser Val Glu Ile
290 295 300

Asn Cys Thr Gly Ala Gly His Cys Asn Ile Ser Arg Ala Lys Trp Asn
305 310 315 320

Ala Thr Leu Lys Gln Ile Ala Ser Lys Leu Arg Glu Gln Phe Gly Asn
325 330 335

Asn Lys Thr Ile Ile Phe Lys Gln Ser Ser Gly Gly Asp Pro Glu Ile
340 345 350

Val Thr His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys Asn Ser
355 360 365

Thr Gln Leu Phe Asn Ser Thr Trp Phe Asn Ser Thr Trp Ser Thr Glu
370 375 380

Gly Ser Asn Asn Thr Glu Gly Ser Asp Thr Ile Thr Leu Pro Cys Arg
385 390 395 400

Ile Lys Gln Phe Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr
405 410 415

Ala Pro Pro Ile Ser Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr Gly
420 425 430

Leu Leu Leu Thr Arg Asp Gly Gly Asn Asn Asn Asn Gly Ser Glu Ile
435 440 445

Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu
450 455 460

Tyr Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Val Ala Pro Thr
465 470 475 480

Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys
485 490

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1448 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1439
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

ATG GAT GCA ATG AAG AGA GGG CTC TGC TGT GTG CTG CTG CTG TGT GGA 48
Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
1 5 10 15

GCA GTC TTC GTT TCG CCC AGC CAG GAA ATC CAT GCC CGA TTC AGA AGA 96
Ala Val Phe Val Ser Pro Ser Gln Glu Ile His Ala Arg Phe Arg Arg
20 25 30

GGC GGC AGA GTA GAA AAG TTG TGG GTC ACA GTC TAT TAT GGG GTA CCT 144
Gly Gly Arg Val Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
35 40 45

GTG TGG AAA GAA GCA ACC ACC ACT CTA TTT TGT GCA TCA GAT GCT AAA 192
Val Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
50 55 60

GCA TAT GAT ACA GAG GTA CAT AAT GTT TGG GCC ACA CAT GCC TGT GTA 240
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
65 70 75 80

CCC ACA GAC CCC AAC CCA CAA GAA GTA GTA TTG GAA AAT GTA ACA GAA 288
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Glu Asn Val Thr Glu
85 90 95

CAT TTT AAC ATG TGG AAA AAT AAC ATG GTA GAA CAG ATG CAG GAG GAT 336
His Phe Asn Met Trp Lys Asn Asn Met Val Glu Gln Met Gln Glu Asp
100 105 110

ATA ATC AGT TTA TGG GAT CAA AGC CTA AAG CCA TGT GTA AAA TTA ACC 384
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
115 120 125

CCA CTC TGT GTT ACT TTA AAT TGC AAG GAT GTG AAT GCT ACT AAT ACC 432
Pro Leu Cys Val Thr Leu Asn Cys Lys Asp Val Asn Ala Thr Asn Thr
130 135 140

ACT AAT GAT AGC GAG GGA ACG ATG GAG AGA GGA GAA ATA AAA AAC TGC 480
Thr Asn Asp Ser Glu Gly Thr Met Glu Arg Gly Glu Ile Lys Asn Cys
145 150 155 160

TCT TTC AAT ATC ACC ACA AGC ATA AGA GAT GAG GTG CAG AAA GAA TAT 528
Ser Phe Asn Ile Thr Thr Ser Ile Arg Asp Glu Val Gln Lys Glu Tyr
165 170 175

GCT CTT TTT TAT AAA CTT GAT GTA GTA CCA ATA GAT AAT AAT AAT ACC 576
Ala Leu Phe Tyr Lys Leu Asp Val Val Pro Ile Asp Asn Asn Asn Thr
180 185 190

AGC TAT AGG TTG ATA AGT TGT GAC ACC TCA GTC ATT ACA CAG GCC TGT 624
Ser Tyr Arg Leu Ile Ser Cys Asp Thr Ser Val Ile Thr Gln Ala Cys
195 200 205

CCA AAG ATA TCC TTT GAG CCA ATT CCC ATA CAT TAT TGT GCC CCG GCT 672
Pro Lys Ile Ser Phe Glu Pro Ile Pro Ile His Tyr Cys Ala Pro Ala
210 215 220

GGT TTT GCG ATT CTA AAG TGT AAT GAT AAG ACG TTC AAT GGA AAA GGA 720
Gly Phe Ala Ile Leu Lys Cys Asn Asp Lys Thr Phe Asn Gly Lys Gly
225 230 235 240

CCA TGT AAA AAT GTC AGC ACA GTA CAA TGT ACA CAT GGA ATT AGG CCA 768
Pro Cys Lys Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro
245 250 255

GTA GTA TCA ACT CAA CTG CTG CTA AAT GGC AGT CTA GCA GAA GAA GAG 816
Val Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu
260 265 270

GTA GTA ATT AGA TCT GAC AAT TTC ACG AAC AAT GCT AAA ACC ATA ATA 864
Val Val Ile Arg Ser Asp Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile
275 280 285

GTA CAG CTG AAA GAA TCT GTA GAA ATT AAT TGT ACA GGT GCT GGA CAT 912
Val Gln Leu Lys Glu Ser Val Glu Ile Asn Cys Thr Gly Ala Gly His
290 295 300

TGT AAC ATT AGT AGA GCA AAA TGG AAT GAC ACT TTA AAA CAG ATA GTT 960
Cys Asn Ile Ser Arg Ala Lys Trp Asn Asp Thr Leu Lys Gln Ile Val
305 310 315 320

ATA AAA TTA AGA GAA CAA TTT GAG AAT AAA ACA ATA GTC TTT AAT CAC 1008

Ile Lys Leu Arg Glu Gln Phe Glu Asn Lys Thr Ile Val Phe Asn His
325 330 335

TCC TCA GGA GGG GAC CCA GAA ATT GTA ATG CAC AGT TTT AAT TGT GGA 1056
Ser Ser Gly Gly Asp Pro Glu Ile Val Met His Ser Phe Asn Cys Gly
340 345 350

GGA GAA TTT TTC TAC TGT AAT TCA ACA CAA CTG TTT AAT AGT ACT TGG 1104
Gly Glu Phe Phe Tyr Cys Asn Ser Thr Gln Leu Phe Asn Ser Thr Trp
355 360 365

AAT AAT AAT ACT GAA GGG TCA AAT AAC ACT GAA GGA AAT ACT ATC ACA 1152
Asn Asn Asn Thr Glu Gly Ser Asn Asn Thr Glu Gly Asn Thr Ile Thr
370 375 380

CTC CCA TGC AGA ATA AAA CAA ATT ATA AAC ATG TGG CAG GAA GTA GGA 1200
Leu Pro Cys Arg Ile Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly
385 390 395 400

AAA GCA ATG TAT GCC CCT CCC ATC AGA GGA CAA ATT AGA TGT TCA TCA 1248
Lys Ala Met Tyr Ala Pro Pro Ile Arg Gly Gln Ile Arg Cys Ser Ser
405 410 415

AAT ATT ACA GGG CTG CTA TTA ACA AGA GAT GGT GGT ATT AAT GAG AAT 1296
Asn Ile Thr Gly Leu Leu Leu Thr Arg Asp Gly Gly Ile Asn Glu Asn
420 425 430

GGG ACC GAG ATC TTC AGA CCT GGA GGA GGA GAT ATG AGG GAC AAT TGG 1344
Gly Thr Glu Ile Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp
435 440 445

AGA AGT GAA TTA TAT AAA TAT AAA GTA GTA AAA ATT GAA CCA TTA GGA 1392
Arg Ser Glu Leu Tyr Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly
450 455 460

GTA GCA CCC ACC AAG GCA AAG AGA AGA GTG GTG CAA AGA GAA AAA TG 1439
Val Ala Pro Thr Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys
465 470 475

AGCGGCCGC 1448

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 479 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
1 5 10 15

Ala Val Phe Val Ser Pro Ser Gln Glu Ile His Ala Arg Phe Arg Arg
20 25 30

Gly Gly Arg Val Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
35 40 45

Val Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
50 55 60

Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
65 70 75 80

Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Glu Asn Val Thr Glu

85 90 95

His Phe Asn Met Trp Lys Asn Asn Met Val Glu Gln Met Gln Glu Asp
100 105 110

Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
115 120 125

Pro Leu Cys Val Thr Leu Asn Cys Lys Asp Val Asn Ala Thr Asn Thr
130 135 140

Thr Asn Asp Ser Glu Gly Thr Met Glu Arg Gly Glu Ile Lys Asn Cys
145 150 155 160

Ser Phe Asn Ile Thr Thr Ser Ile Arg Asp Glu Val Gln Lys Glu Tyr
165 170 175

Ala Leu Phe Tyr Lys Leu Asp Val Val Pro Ile Asp Asn Asn Asn Thr
180 185 190

Ser Tyr Arg Leu Ile Ser Cys Asp Thr Ser Val Ile Thr Gln Ala Cys
195 200 205

Pro Lys Ile Ser Phe Glu Pro Ile Pro Ile His Tyr Cys Ala Pro Ala
210 215 220

Gly Phe Ala Ile Leu Lys Cys Asn Asp Lys Thr Phe Asn Gly Lys Gly
225 230 235 240

Pro Cys Lys Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro
245 250 255

Val Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu
260 265 270

Val Val Ile Arg Ser Asp Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile
275 280 285

Val Gln Leu Lys Glu Ser Val Glu Ile Asn Cys Thr Gly Ala Gly His
290 295 300

Cys Asn Ile Ser Arg Ala Lys Trp Asn Asp Thr Leu Lys Gln Ile Val
305 310 315 320

Ile Lys Leu Arg Glu Gln Phe Glu Asn Lys Thr Ile Val Phe Asn His
325 330 335

Ser Ser Gly Gly Asp Pro Glu Ile Val Met His Ser Phe Asn Cys Gly
340 345 350

Gly Glu Phe Phe Tyr Cys Asn Ser Thr Gln Leu Phe Asn Ser Thr Trp
355 360 365

Asn Asn Asn Thr Glu Gly Ser Asn Asn Thr Glu Gly Asn Thr Ile Thr
370 375 380

Leu Pro Cys Arg Ile Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly
385 390 395 400

Lys Ala Met Tyr Ala Pro Pro Ile Arg Gly Gln Ile Arg Cys Ser Ser
405 410 415

Asn Ile Thr Gly Leu Leu Leu Thr Arg Asp Gly Gly Ile Asn Glu Asn
420 425 430

Gly Thr Glu Ile Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp
435 440 445

Arg Ser Glu Leu Tyr Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly

450 455 460

Val Ala Pro Thr Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys
465 470 475

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1484 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1454
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

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ATG GAT GCA ATG AAG AGA GGG CTC TGC TGT GTG CTG CTG CTG TGT GGA      48
Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
  1               5                  10                  15

GCA GTC TTC GTT TCG CCC AGC CAG GAA ATC CAT GCC CGA TTC AGA AGA      96
Ala Val Phe Val Ser Pro Ser Gln Glu Ile His Ala Arg Phe Arg Arg
             20                  25                  30

GGC GCC AGA ACA GAA AAA TTG TGG GTC ACA GTC TAT TAT GGG GTA CCT     144
Gly Ala Arg Thr Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
         35                  40                  45

GTG TGG AAG GAA GCA ACC ACC ACT CTA TTT TGT GCA TCA GAT GCT AAA     192
Val Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
     50                  55                  60

GCA TAT GAT ACA GAG GTA CAT AAT GTT TGG GCC ACA CAT GCC TGT GTA     240
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
 65                  70                  75                  80

CCC ACA GAC CCC AAC CCA CAA GAA GTA GTA TTG GTA AAT GTG ACA GAA     288
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Val Asn Val Thr Glu
                 85                  90                  95

AAT TTT AAC ATG TGG AAA AAT GAC ATG GTA GAA CAG ATG CAT GAG GAT     336
Asn Phe Asn Met Trp Lys Asn Asp Met Val Glu Gln Met His Glu Asp
            100                 105                 110

ATA ATC AGT TTA TGG GAT CAA AGC CTA AAG CCA TGT GTA AAA TTA ACC     384
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
        115                 120                 125

CCA CTC TGT GTT AGT TTA AAG TGC ACT GAT TTG GGG AAT GCT ACT AAT     432
Pro Leu Cys Val Ser Leu Lys Cys Thr Asp Leu Gly Asn Ala Thr Asn
    130                 135                 140

ACC AAT AGT AGT AAT ACC AAT AGT AGT AGC GGG GAA ATG ATG ATG GAG     480
Thr Asn Ser Ser Asn Thr Asn Ser Ser Ser Gly Glu Met Met Met Glu
145                 150                 155                 160

AAA GGA GAG ATA AAA AAC TGC TCT TTC AAT ATC AGC ACA AGC ATA AGA     528
Lys Gly Glu Ile Lys Asn Cys Ser Phe Asn Ile Ser Thr Ser Ile Arg
                165                 170                 175

GGT AAG GTG CAG AAA GAA TAT GCA TTT TTT TAT AAA CTT GAT ATA ATA     576
Gly Lys Val Gln Lys Glu Tyr Ala Phe Phe Tyr Lys Leu Asp Ile Ile
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        180                 185                 190

 CCA ATA GAT AAT GAT ACT ACC AGC TAT ACG TTG ACA AGT TGT AAC ACC       624
 Pro Ile Asp Asn Asp Thr Thr Ser Tyr Thr Leu Thr Ser Cys Asn Thr
         195                 200                 205

 TCA GTC ATT ACA CAG GCC TGT CCA AAG GTA TCC TTT GAG CCA ATT CCC       672
 Ser Val Ile Thr Gln Ala Cys Pro Lys Val Ser Phe Glu Pro Ile Pro
     210                 215                 220

 ATA CAT TAT TGT GCC CCG GCT GGT TTT GCG ATT CTA AAA TGT AAT AAT       720
 Ile His Tyr Cys Ala Pro Ala Gly Phe Ala Ile Leu Lys Cys Asn Asn
 225                 230                 235                 240

 AAG ACG TTC AAT GGA ACA GGA CCA TGT ACA AAT GTC AGC ACA GTA CAA       768
 Lys Thr Phe Asn Gly Thr Gly Pro Cys Thr Asn Val Ser Thr Val Gln
                 245                 250                 255

 TGT ACA CAT GGA ATT AGG CCA GTA GTA TCA ACT CAA CTG CTG TTG AAT       816
 Cys Thr His Gly Ile Arg Pro Val Val Ser Thr Gln Leu Leu Leu Asn
             260                 265                 270

 GGC ACT CTA GCA GAA GAA GAG GTA GTA ATT AGA TCT GCC AAT TTC ACA       864
 Gly Ser Leu Ala Glu Glu Glu Val Val Ile Arg Ser Ala Asn Phe Thr
         275                 280                 285

 GAC AAT GCT AAA ACC ATA ATA GTA CAG CTG AAC CAA TCT GTA GAA ATT       912
 Asp Asn Ala Lys Thr Ile Ile Val Gln Leu Asn Gln Ser Val Glu Ile
     290                 295                 300

 AAT TGT ACA GGT GCT GGA CAT TGT AAC ATT AGT AGA GCA AAA TGG AAT       960
 Asn Cys Thr Gly Ala Gly His Cys Asn Ile Ser Arg Ala Lys Trp Asn
 305                 310                 315                 320

 GCC ACT TTA AAA CAG ATA GCT AGC AAA TTA AGA GAA CAA TTT GGA AAT      1008
 Ala Thr Leu Lys Gln Ile Ala Ser Lys Leu Arg Glu Gln Phe Gly Asn
                 325                 330                 335

 AAT AAA ACA ATA ATC TTT AAG CAA TCC TCA GGA GGG GAC CCA GAA ATT      1056
 Asn Lys Thr Ile Ile Phe Lys Gln Ser Ser Gly Gly Asp Pro Glu Ile
             340                 345                 350

 GTA ACG CAC AGT TTT AAT TGT GGA GGG GAA TTT TTC TAC TGT AAT TCA      1104
 Val Thr His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys Asn Ser
         355                 360                 365

 ACA CAA CTG TTT AAT AGT ACT TGG TTT AAT AGT ACT TGG AGT ACT GAA      1152
 Thr Gln Leu Phe Asn Ser Thr Trp Phe Asn Ser Thr Trp Ser Thr Glu
     370                 375                 380

 GGG TCA AAT AAC ACT GAA GGA AGT GAC ACA ATC ACA CTC CCA TGC AGA      1200
 Gly Ser Asn Asn Thr Glu Gly Ser Asp Thr Ile Thr Leu Pro Cys Arg
 385                 390                 395                 400

 ATA AAA CAA TTT ATA AAC ATG GTG CAG GAA GTA GGA AAA GCA ATG TAT      1248
 Ile Lys Gln Phe Ile Asn Met Val Gln Glu Val Gly Lys Ala Met Tyr
                 405                 410                 415

 GCC CCT CCC ATC AGC GGA CAA ATT AGA TGT TCA TCA AAT ATT ACA GGG      1296
 Ala Pro Pro Ile Ser Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr Gly
             420                 425                 430

 CTG CTA TTA ACA AGA GAT GGT GGT AAT AAC AAC AAT GGG TCC GAG ATC      1344
 Leu Leu Leu Thr Arg Asp Gly Gly Asn Asn Asn Asn Gly Ser Glu Ile
         435                 440                 445

 TTC AGA CCT GGA GGA GGA GAT ATG AGG GAC AAT TGG AGA AGT GAA TTA      1392
 Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu
     450                 455                 460
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TAT AAA TAT AAA GTA GTA AAA ATT GAA CCA TTA GGA GTA GCA CCC ACC        1440
Tyr Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Val Ala Pro Thr
465                 470                 475                 480

AAG GCA AAG AGA AG AGTGGTGCAG AGAGAAAAAT GAGCGGCCGC                    1484
Lys Ala Lys Arg
```

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 484 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
1               5                   10                  15

Ala Val Phe Val Ser Pro Ser Gln Glu Ile His Ala Arg Phe Arg Arg
            20                  25                  30

Gly Ala Arg Thr Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
        35                  40                  45

Val Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
    50                  55                  60

Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
65                  70                  75                  80

Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Val Asn Val Thr Glu
                85                  90                  95

Asn Phe Asn Met Trp Lys Asn Asp Met Val Glu Gln Met His Glu Asp
            100                 105                 110

Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
        115                 120                 125

Pro Leu Cys Val Ser Leu Lys Cys Thr Asp Leu Gly Asn Ala Thr Asn
    130                 135                 140

Thr Asn Ser Ser Asn Thr Asn Ser Ser Ser Gly Glu Met Met Met Glu
145                 150                 155                 160

Lys Gly Glu Ile Lys Asn Cys Ser Phe Asn Ile Ser Thr Ser Ile Arg
                165                 170                 175

Gly Lys Val Gln Lys Glu Tyr Ala Phe Phe Tyr Lys Leu Asp Ile Ile
            180                 185                 190

Pro Ile Asp Asn Asp Thr Thr Ser Tyr Thr Leu Thr Ser Cys Asn Thr
        195                 200                 205

Ser Val Ile Thr Gln Ala Cys Pro Lys Val Ser Phe Glu Pro Ile Pro
    210                 215                 220

Ile His Tyr Cys Ala Pro Ala Gly Phe Ala Ile Leu Lys Cys Asn Asn
225                 230                 235                 240

Lys Thr Phe Asn Gly Thr Gly Pro Cys Thr Asn Val Ser Thr Val Gln
                245                 250                 255

Cys Thr His Gly Ile Arg Pro Val Val Ser Thr Gln Leu Leu Leu Asn
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260 265 270

Gly Ser Leu Ala Glu Glu Glu Val Val Ile Arg Ser Ala Asn Phe Thr
275 280 285

Asp Asn Ala Lys Thr Ile Ile Val Gln Leu Asn Gln Ser Val Glu Ile
290 295 300

Asn Cys Thr Gly Ala Gly His Cys Asn Ile Ser Arg Ala Lys Trp Asn
305 310 315 320

Ala Thr Leu Lys Gln Ile Ala Ser Lys Leu Arg Glu Gln Phe Gly Asn
325 330 335

Asn Lys Thr Ile Ile Phe Lys Gln Ser Ser Gly Gly Asp Pro Glu Ile
340 345 350

Val Thr His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys Asn Ser
355 360 365

Thr Gln Leu Phe Asn Ser Thr Trp Phe Asn Ser Thr Trp Ser Thr Glu
370 375 380

Gly Ser Asn Asn Thr Glu Gly Ser Asp Thr Ile Thr Leu Pro Cys Arg
385 390 395 400

Ile Lys Gln Phe Ile Asn Met Val Gln Glu Val Gly Lys Ala Met Tyr
405 410 415

Ala Pro Pro Ile Ser Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr Gly
420 425 430

Leu Leu Leu Thr Arg Asp Gly Gly Asn Asn Asn Asn Gly Ser Glu Ile
435 440 445

Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu
450 455 460

Tyr Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Val Ala Pro Thr
465 470 475 480

Lys Ala Lys Arg

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1448 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1438
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

ATG GAT GCA ATG AAG AGA GGG CTC TGC TGT GTG CTG CTG CTG TGT GGA 48
Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
1 5 10 15

GCA GTC TTC GTT TCG CCC AGC CAG GAA ATC CAT GCC CGA TTC AGA AGA 96
Ala Val Phe Val Ser Pro Ser Gln Glu Ile His Ala Arg Phe Arg Arg
20 25 30

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GGC GGC AGA GTA GAA AAG TTG TGG GTC ACA GTC TAT TAT GGG GTA CCT     144
Gly Gly Arg Val Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
        35                  40                  45

GTG TGG AAA GAA GCA ACC ACC ACT CTA TTT TGT GCA TCA GAT GCT AAA     192
Val Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
    50                  55                  60

GCA TAT GAT ACA GAG GTA CAT AAT GTT TGG GCC ACA CAT GCC TGT GTA     240
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
65                  70                  75                  80

CCC ACA GAC CCC AAC CCA CAA GAA GTA GTA TTG GAA AAT GTA ACA GAA     288
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Glu Asn Val Thr Glu
                85                  90                  95

CAT TTT AAC ATG TGG AAA AAT AAC ATG GTA GAA CAG ATG CAG GAG GAT     336
His Phe Asn Met Trp Lys Asn Asn Met Val Glu Gln Met Gln Glu Asp
            100                 105                 110

ATA ATC AGT TTA TGG GAT CAA AGC CTA AAG CCA TGT GTA AAA TTA ACC     384
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
        115                 120                 125

CCA CTC TGT GTT ACT TTA AAT TGC AAG GAT GTG AAT GCT ACT AAT ACC     432
Pro Leu Cys Val Thr Leu Asn Cys Lys Asp Val Asn Ala Thr Asn Thr
    130                 135                 140

ACT AAT GAT AGC GAG GGA ACG ATG GAG AGA GGA GAA ATA AAA AAC TGC     480
Thr Asn Asp Ser Glu Gly Thr Met Glu Arg Gly Glu Ile Lys Asn Cys
145                 150                 155                 160

TCT TTC AAT ATC ACC ACA AGC ATA AGA GAT GAG GTG CAG AAA GAA TAT     528
Ser Phe Asn Ile Thr Thr Ser Ile Arg Asp Glu Val Gln Lys Glu Tyr
                165                 170                 175

GCT CTT TTT TAT AAA CTT GAT GTA GTA CCA ATA GAT AAT AAT AAT ACC     576
Ala Leu Phe Tyr Lys Leu Asp Val Val Pro Ile Asp Asn Asn Asn Thr
            180                 185                 190

AGC TAT AGG TTG ATA AGT TGT GAC ACC TCA GTC ATT ACA CAG GCC TGT     624
Ser Tyr Arg Leu Ile Ser Cys Asp Thr Ser Val Ile Thr Gln Ala Cys
        195                 200                 205

CCA AAG ATA TCC TTT GAG CCA ATT CCC ATA CAT TAT TGT GCC CCG GCT     672
Pro Lys Ile Ser Phe Glu Pro Ile Pro Ile His Tyr Cys Ala Pro Ala
    210                 215                 220

GGT TTT GCG ATT CTA AAG TGT AAT GAT AAG ACG TTC AAT GGA AAA GGA     720
Gly Phe Ala Ile Leu Lys Cys Asn Asp Lys Thr Phe Asn Gly Lys Gly
225                 230                 235                 240

CCA TGT AAA AAT GTC AGC ACA GTA CAA TGT ACA CAT GGA ATT AGG CCA     768
Pro Cys Lys Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro
                245                 250                 255

GTA GTA TCA ACT CAA CTG CTG CTA AAT GGC AGT CTA GCA GAA GAA GAG     816
Val Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu
            260                 265                 270

GTA GTA ATT AGA TCT GAC AAT TTC ACG AAC AAT GCT AAA ACC ATA ATA     864
Val Val Ile Arg Ser Asp Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile
        275                 280                 285

GTA CAG CTG AAA GAA TCT GTA GAA ATT AAT TGT ACA GGT GCT GGA CAT     912
Val Gln Leu Lys Glu Ser Val Glu Ile Asn Cys Thr Gly Ala Gly His
    290                 295                 300

TGT AAC ATT AGT AGA GCA AAA TGG AAT GAC ACT TTA AAA CAG ATA GTT     960
```

Cys Asn Ile Ser Arg Ala Lys Trp Asn Asp Thr Leu Lys Gln Ile Val
305 310 315 320

ATA AAA TTA AGA GAA CAA TTT GAG AAT AAA ACA ATA GTC TTT AAT CAC 1008
Ile Lys Leu Arg Glu Gln Phe Glu Asn Lys Thr Ile Val Phe Asn His
325 330 335

TCC TCA GGA GGG GAC CCA GAA ATT GTA ATG CAC AGT TTT AAT TGT GGA 1056
Ser Ser Gly Gly Asp Pro Glu Ile Val Met His Ser Phe Asn Cys Gly
340 345 350

GGA GAA TTT TTC TAC TGT AAT TCA ACA CAA CTG TTT AAT AGT ACT TGG 1104
Gly Glu Phe Phe Tyr Cys Asn Ser Thr Gln Leu Phe Asn Ser Thr Trp
355 360 365

AAT AAT AAT ACT GAA GGG TCA AAT AAC ACT GAA GGA AAT ACT ATC ACA 1152
Asn Asn Asn Thr Glu Gly Ser Asn Asn Thr Glu Gly Asn Thr Ile Thr
370 375 380

CTC CCA TGC AGA ATA AAA CAA ATT ATA AAC ATG GTG CAG GAA GTA GGA 1200
Leu Pro Cys Arg Ile Lys Gln Ile Ile Asn Met Val Gln Glu Val Gly
385 390 395 400

AAA GCA ATG TAT GCC CCT CCC ATC AGA GGA CAA ATT AGA TGT TCA TCA 1248
Lys Ala Met Tyr Ala Pro Pro Ile Arg Gly Gln Ile Arg Cys Ser Ser
405 410 415

AAT ATT ACA GGG CTG CTA TTA ACA AGA GAT GGT GGT ATT AAT GAG AAT 1296
Asn Ile Thr Gly Leu Leu Leu Thr Arg Asp Gly Gly Ile Asn Glu Asn
420 425 430

GGG ACC GAG ATC TTC AGA CCT GGA GGA GGA GAT ATG AGG GAC AAT TGG 1344
Gly Thr Glu Ile Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp
435 440 445

AGA AGT GAA TTA TAT AAA TAT AAA GTA GTA AAA ATT GAA CCA TTA GGA 1392
Arg Ser Glu Leu Tyr Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly
450 455 460

GTA GCA CCC ACC AAG GCA AAG AGA AGA GTG GTG CAA AGA GAA AAA T 1438
Val Ala Pro Thr Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys
465 470 475

GAGCGGCCGC 1448

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 479 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
1 5 10 15

Ala Val Phe Val Ser Pro Ser Gln Glu Ile His Ala Arg Phe Arg Arg
20 25 30

Gly Gly Arg Val Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
35 40 45

Val Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
50 55 60

Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
65 70 75 80

Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Glu Asn Val Thr Glu
85 90 95

His Phe Asn Met Trp Lys Asn Asn Met Val Glu Gln Met Gln Glu Asp
100 105 110

Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
115 120 125

Pro Leu Cys Val Thr Leu Asn Cys Lys Asp Val Asn Ala Thr Asn Thr
130 135 140

Thr Asn Asp Ser Glu Gly Thr Met Glu Arg Gly Glu Ile Lys Asn Cys
145 150 155 160

Ser Phe Asn Ile Thr Thr Ser Ile Arg Asp Glu Val Gln Lys Glu Tyr
165 170 175

Ala Leu Phe Tyr Lys Leu Asp Val Val Pro Ile Asp Asn Asn Asn Thr
180 185 190

Ser Tyr Arg Leu Ile Ser Cys Asp Thr Ser Val Ile Thr Gln Ala Cys
195 200 205

Pro Lys Ile Ser Phe Glu Pro Ile Pro Ile His Tyr Cys Ala Pro Ala
210 215 220

Gly Phe Ala Ile Leu Lys Cys Asn Asp Lys Thr Phe Asn Gly Lys Gly
225 230 235 240

Pro Cys Lys Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro
245 250 255

Val Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu
260 265 270

Val Val Ile Arg Ser Asp Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile
275 280 285

Val Gln Leu Lys Glu Ser Val Glu Ile Asn Cys Thr Gly Ala Gly His
290 295 300

Cys Asn Ile Ser Arg Ala Lys Trp Asn Asp Thr Leu Lys Gln Ile Val
305 310 315 320

Ile Lys Leu Arg Glu Gln Phe Glu Asn Lys Thr Ile Val Phe Asn His
325 330 335

Ser Ser Gly Gly Asp Pro Glu Ile Val Met His Ser Phe Asn Cys Gly
340 345 350

Gly Glu Phe Phe Tyr Cys Asn Ser Thr Gln Leu Phe Asn Ser Thr Trp
355 360 365

Asn Asn Asn Thr Glu Gly Ser Asn Asn Thr Glu Gly Asn Thr Ile Thr
370 375 380

Leu Pro Cys Arg Ile Lys Gln Ile Ile Asn Met Val Gln Glu Val Gly
385 390 395 400

Lys Ala Met Tyr Ala Pro Pro Ile Arg Gly Gln Ile Arg Cys Ser Ser
405 410 415

Asn Ile Thr Gly Leu Leu Leu Thr Arg Asp Gly Gly Ile Asn Glu Asn
420 425 430

```
Gly Thr Glu Ile Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp
        435                 440                 445

Arg Ser Glu Leu Tyr Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly
    450                 455                 460

Val Ala Pro Thr Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys
465                 470                 475
```

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1571 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1567
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
ATG GAT GCA ATG AAG AGA GGG CTC TGC TGT GTG CTG CTG CTG TGT GGA       48
Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
  1               5                  10                  15

GCA GTC TTC GTT TCG CCC AGC CAG GAA ATC CAT GCC CGA TTC AGA AGA       96
Ala Val Phe Val Ser Pro Ser Gln Glu Ile His Ala Arg Phe Arg Arg
             20                  25                  30

GGC GCC AGA ACA GAA AAA TTG TGG GTC ACA GTC TAT TAT GGG GTA CCT      144
Gly Ala Arg Thr Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
         35                  40                  45

GTG TGG AAG GAA GCA ACC ACC ACT CTA TTT TGT GCA TCA GAT GCT AAA      192
Val Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
     50                  55                  60

GCA TAT GAT ACA GAG GTA CAT AAT GTT TGG GCC ACA CAT GCC TGT GTA      240
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
 65                  70                  75                  80

CCC ACA GAC CCC AAC CCA CAA GAA GTA GTA TTG GTA AAT GTG ACA GAA      288
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Val Asn Val Thr Glu
                 85                  90                  95

AAT TTT AAC ATG TGG AAA AAT GAC ATG GTA GAA CAG ATG CAT GAG GAT      336
Asn Phe Asn Met Trp Lys Asn Asp Met Val Glu Gln Met His Glu Asp
            100                 105                 110

ATA ATC AGT TTA TGG GAT CAA AGC CTA AAG CCA TGT GTA AAA TTA ACC      384
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
        115                 120                 125

CCA CTC TGT GTT AGT TTA AAG TGC ACT GAT TTG GGG AAT GCT ACT AAT      432
Pro Leu Cys Val Ser Leu Lys Cys Thr Asp Leu Gly Asn Ala Thr Asn
    130                 135                 140

ACC AAT AGT AGT AAT ACC AAT AGT AGT AGC GGG GAA ATG ATG ATG GAG      480
Thr Asn Ser Ser Asn Thr Asn Ser Ser Ser Gly Glu Met Met Met Glu
145                 150                 155                 160

AAA GGA GAG ATA AAA AAC TGC TCT TTC AAT ATC AGC ACA AGC ATA AGA      528
Lys Gly Glu Ile Lys Asn Cys Ser Phe Asn Ile Ser Thr Ser Ile Arg
```

165 170 175

GGT AAG GTG CAG AAA GAA TAT GCA TTT TTT TAT AAA CTT GAT ATA ATA 576
Gly Lys Val Gln Lys Glu Tyr Ala Phe Phe Tyr Lys Leu Asp Ile Ile
180 185 190

CCA ATA GAT AAT GAT ACT ACC AGC TAT ACG TTG ACA AGT TGT AAC ACC 624
Pro Ile Asp Asn Asp Thr Thr Ser Tyr Thr Leu Thr Ser Cys Asn Thr
195 200 205

TCA GTC ATT ACA CAG GCC TGT CCA AAG GTA TCC TTT GAG CCA ATT CCC 672
Ser Val Ile Thr Gln Ala Cys Pro Lys Val Ser Phe Glu Pro Ile Pro
210 215 220

ATA CAT TAT TGT GCC CCG GCT GGT TTT GCG ATT CTA AAA TGT AAT AAT 720
Ile His Tyr Cys Ala Pro Ala Gly Phe Ala Ile Leu Lys Cys Asn Asn
225 230 235 240

AAG ACG TTC AAT GGA ACA GGA CCA TGT ACA AAT GTC AGC ACA GTA CAA 768
Lys Thr Phe Asn Gly Thr Gly Pro Cys Thr Asn Val Ser Thr Val Gln
245 250 255

TGT ACA CAT GGA ATT AGG CCA GTA GTA TCA ACT CAA CTG CTG TTG AAT 816
Cys Thr His Gly Ile Arg Pro Val Val Ser Thr Gln Leu Leu Leu Asn
260 265 270

GGC AGT CTA GCA GAA GAA GAG GTA GTA ATT AGA TCT GCC AAT TTC ACA 864
Gly Ser Leu Ala Glu Glu Glu Val Val Ile Arg Ser Ala Asn Phe Thr
275 280 285

GAC AAT GCT AAA ACC ATA ATA GTA CAG CTG AAC CAA TCT GTA GAA ATT 912
Asp Asn Ala Lys Thr Ile Ile Val Gln Leu Asn Gln Ser Val Glu Ile
290 295 300

AAT TGT ACA AGA CCC AAC AAC AAT ACA AGA AAA AGT ATC CGT ATC CAG 960
Asn Cys Thr Arg Pro Asn Asn Asn Thr Arg Lys Ser Ile Arg Ile Gln
305 310 315 320

AGG GGA CCA GGG AGA GCA TTT GTT ACA ATA GGA AAA ATA GGA AAT ATG 1008
Arg Gly Pro Gly Arg Ala Phe Val Thr Ile Gly Lys Ile Gly Asn Met
325 330 335

AGA CAA GCA CAT TGT AAC ATT AGT AGA GCA AAA TGG AAT GCC ACT TTA 1056
Arg Gln Ala His Cys Asn Ile Ser Arg Ala Lys Trp Asn Ala Thr Leu
340 345 350

AAA CAG ATA GCT AGC AAA TTA AGA GAA CAA TTT GGA AAT AAT AAA ACA 1104
Lys Gln Ile Ala Ser Lys Leu Arg Glu Gln Phe Gly Asn Asn Lys Thr
355 360 365

ATA ATC TTT AAG CAA TCC TCA GGA GGG GAC CCA GAA ATT GTA ACG CAC 1152
Ile Ile Phe Lys Gln Ser Ser Gly Gly Asp Pro Glu Ile Val Thr His
370 375 380

AGT TTT AAT TGT GGA GGG GAA TTT TTC TAC TGT AAT TCA ACA CAA CTG 1200
Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys Asn Ser Thr Gln Leu
385 390 395 400

TTT AAT AGT ACT TGG TTT AAT AGT ACT TGG AGT ACT GAA GGG TCA AAT 1248
Phe Asn Ser Thr Trp Phe Asn Ser Thr Trp Ser Thr Glu Gly Ser Asn
405 410 415

AAC ACT GAA GGA AGT GAC ACA ATC ACA CTC CCA TGC AGA ATA AAA CAA 1296
Asn Thr Glu Gly Ser Asp Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln
420 425 430

TTT ATA AAC ATG GTG CAG GAA GTA GGA AAA GCA ATG TAT GCC CCT CCC 1344
Phe Ile Asn Met Val Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro
435 440 445

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ATC AGC GGA CAA ATT AGA TGT TCA TCA AAT ATT ACA GGG CTG CTA TTA      1392
Ile Ser Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr Gly Leu Leu Leu
    450                 455                 460

ACA AGA GAT GGT GGT AAT AAC AAC AAT GGG TCC GAG ATC TTC AGA CCT      1440
Thr Arg Asp Gly Gly Asn Asn Asn Asn Gly Ser Glu Ile Phe Arg Pro
465                 470                 475                 480

GGA GGA GGA GAT ATG AGG GAC AAT TGG AGA AGT GAA TTA TAT AAA TAT      1488
Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr Lys Tyr
                485                 490                 495

AAA GTA GTA AAA ATT GAA CCA TTA GGA GTA GCA CCC ACC AAG GCA AAG      1536
Lys Val Val Lys Ile Glu Pro Leu Gly Val Ala Pro Thr Lys Ala Lys
            500                 505                 510

AGA AGA GTG GTG CAG AGA GAA AAA TGA GCG G CCGC                       1571
Arg Arg Val Val Gln Arg Glu Lys
        515                 520
```

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 522 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

```
Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
1               5                   10                  15

Ala Val Phe Val Ser Pro Ser Gln Glu Ile His Ala Arg Phe Arg Arg
            20                  25                  30

Gly Ala Arg Thr Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
        35                  40                  45

Val Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
    50                  55                  60

Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
65                  70                  75                  80

Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Val Asn Val Thr Glu
                85                  90                  95

Asn Phe Asn Met Trp Lys Asn Asp Met Val Glu Gln Met His Glu Asp
            100                 105                 110

Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
        115                 120                 125

Pro Leu Cys Val Ser Leu Lys Cys Thr Asp Leu Gly Asn Ala Thr Asn
    130                 135                 140

Thr Asn Ser Ser Asn Thr Asn Ser Ser Ser Gly Glu Met Met Met Glu
145                 150                 155                 160

Lys Gly Glu Ile Lys Asn Cys Ser Phe Asn Ile Ser Thr Ser Ile Arg
                165                 170                 175

Gly Lys Val Gln Lys Glu Tyr Ala Phe Phe Tyr Lys Leu Asp Ile Ile
            180                 185                 190

Pro Ile Asp Asn Asp Thr Thr Ser Tyr Thr Leu Thr Ser Cys Asn Thr
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        195                 200                 205
Ser Val Ile Thr Gln Ala Cys Pro Lys Val Ser Phe Glu Pro Ile Pro
    210                 215                 220

Ile His Tyr Cys Ala Pro Ala Gly Phe Ala Ile Leu Lys Cys Asn Asn
225                 230                 235                 240

Lys Thr Phe Asn Gly Thr Gly Pro Cys Thr Asn Val Ser Thr Val Gln
                245                 250                 255

Cys Thr His Gly Ile Arg Pro Val Val Ser Thr Gln Leu Leu Leu Asn
            260                 265                 270

Gly Ser Leu Ala Glu Glu Glu Val Val Ile Arg Ser Ala Asn Phe Thr
        275                 280                 285

Asp Asn Ala Lys Thr Ile Ile Val Gln Leu Asn Gln Ser Val Glu Ile
    290                 295                 300

Asn Cys Thr Arg Pro Asn Asn Asn Thr Arg Lys Ser Ile Arg Ile Gln
305                 310                 315                 320

Arg Gly Pro Gly Arg Ala Phe Val Thr Ile Gly Lys Ile Gly Asn Met
                325                 330                 335

Arg Gln Ala His Cys Asn Ile Ser Arg Ala Lys Trp Asn Ala Thr Leu
            340                 345                 350

Lys Gln Ile Ala Ser Lys Leu Arg Glu Gln Phe Gly Asn Asn Lys Thr
        355                 360                 365

Ile Ile Phe Lys Gln Ser Ser Gly Gly Asp Pro Glu Ile Val Thr His
    370                 375                 380

Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys Asn Ser Thr Gln Leu
385                 390                 395                 400

Phe Asn Ser Thr Trp Phe Asn Ser Thr Trp Ser Thr Glu Gly Ser Asn
                405                 410                 415

Asn Thr Glu Gly Ser Asp Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln
            420                 425                 430

Phe Ile Asn Met Val Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro
        435                 440                 445

Ile Ser Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr Gly Leu Leu Leu
    450                 455                 460

Thr Arg Asp Gly Gly Asn Asn Asn Asn Gly Ser Glu Ile Phe Arg Pro
465                 470                 475                 480

Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr Lys Tyr
                485                 490                 495

Lys Val Val Lys Ile Glu Pro Leu Gly Val Ala Pro Thr Lys Ala Lys
            500                 505                 510

Arg Arg Val Val Gln Arg Glu Lys
        515                 520
```

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1532 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1522
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

ATG GAT GCA ATG AAG AGA GGG CTC TGC TGT GTG CTG CTG CTG TGT GGA 48
Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
1 5 10 15

GCA GTC TTC GTT TCG CCC AGC CAG GAA ATC CAT GCC CGA TTC AGA AGA 96
Ala Val Phe Val Ser Pro Ser Gln Glu Ile His Ala Arg Phe Arg Arg
20 25 30

GGC GGC AGA GTA GAA AAG TTG TGG GTC ACA GTC TAT TAT GGG GTA CCT 144
Gly Gly Arg Val Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
35 40 45

GTG TGG AAA GAA GCA ACC ACC ACT CTA TTT TGT GCA TCA GAT GCT AAA 192
Val Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
50 55 60

GCA TAT GAT ACA GAG GTA CAT AAT GTT TGG GCC ACA CAT GCC TGT GTA 240
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
65 70 75 80

CCC ACA GAC CCC AAC CCA CAA GAA GTA GTA TTG GAA AAT GTA ACA GAA 288
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Glu Asn Val Thr Glu
85 90 95

CAT TTT AAC ATG TGG AAA AAT AAC ATG GTA GAA CAG ATG CAG GAG GAT 336
His Phe Asn Met Trp Lys Asn Asn Met Val Glu Gln Met Gln Glu Asp
100 105 110

ATA ATC AGT TTA TGG GAT CAA AGC CTA AAG CCA TGT GTA AAA TTA ACC 384
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
115 120 125

CCA CTC TGT GTT ACT TTA AAT TGC AAG GAT GTG AAT GCT ACT AAT ACC 432
Pro Leu Cys Val Thr Leu Asn Cys Lys Asp Val Asn Ala Thr Asn Thr
130 135 140

ACT AAT GAT AGC GAG GGA ACG ATG GAG AGA GGA GAA ATA AAA AAC TGC 480
Thr Asn Asp Ser Glu Gly Thr Met Glu Arg Gly Glu Ile Lys Asn Cys
145 150 155 160

TCT TTC AAT ATC ACC ACA AGC ATA AGA GAT GAG GTG CAG AAA GAA TAT 528
Ser Phe Asn Ile Thr Thr Ser Ile Arg Asp Glu Val Gln Lys Glu Tyr
165 170 175

GCT CTT TTT TAT AAA CTT GAT GTA GTA CCA ATA GAT AAT AAT AAT ACC 576
Ala Leu Phe Tyr Lys Leu Asp Val Val Pro Ile Asp Asn Asn Asn Thr
180 185 190

AGC TAT AGG TTG ATA AGT TGT GAC ACC TCA GTC ATT ACA CAG GCC TGT 624
Ser Tyr Arg Leu Ile Ser Cys Asp Thr Ser Val Ile Thr Gln Ala Cys
195 200 205

CCA AAG ATA TCC TTT GAG CCA ATT CCC ATA CAT TAT TGT GCC CCG GCT 672
Pro Lys Ile Ser Phe Glu Pro Ile Pro Ile His Tyr Cys Ala Pro Ala
210 215 220

GGT TTT GCG ATT CTA AAG TGT AAT GAT AAG ACG TTC AAT GGA AAA GGA 720
Gly Phe Ala Ile Leu Lys Cys Asn Asp Lys Thr Phe Asn Gly Lys Gly
225 230 235 240

```
CCA TGT AAA AAT GTC AGC ACA GTA CAA TGT ACA CAT GGA ATT AGG CCA    768
Pro Cys Lys Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro
                245                 250                 255

GTA GTA TCA ACT CAA CTG CTG CTA AAT GGC AGT CTA GCA GAA GAA GAG    816
Val Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu
        260                 265                 270

GTA GTA ATT AGA TCT GAC AAT TTC ACG AAC AAT GCT AAA ACC ATA ATA    864
Val Val Ile Arg Ser Asp Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile
    275                 280                 285

GTA CAG CTG AAA GAA TCT GTA GAA ATT AAT TGT ACA AGA CCC AAC AAC    912
Val Gln Leu Lys Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn
    290                 295                 300

AAT ACA AGA AAA AGT ATA CAT ATA GGA CCA GGG AGA GCA TTT TAT ACT    960
Asn Thr Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr
305                 310                 315                 320

ACA GGA GAA ATA ATA GGA GAT ATA AGA CAA GCA CAT TGT AAC ATT AGT    1008
Thr Gly Glu Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser
                325                 330                 335

AGA GCA AAA TGG AAT GAC ACT TTA AAA CAG ATA GTT ATA AAA TTA AGA    1056
Arg Ala Lys Trp Asn Asp Thr Leu Lys Gln Ile Val Ile Lys Leu Arg
            340                 345                 350

GAA CAA TTT GAG AAT AAA ACA ATA GTC TTT AAT CAC TCC TCA GGA GGG    1104
Glu Gln Phe Glu Asn Lys Thr Ile Val Phe Asn His Ser Ser Gly Gly
        355                 360                 365

GAC CCA GAA ATT GTA ATG CAC AGT TTT AAT TGT GGA GGA GAA TTT TTC    1152
Asp Pro Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe
    370                 375                 380

TAC TGT AAT TCA ACA CAA CTG TTT AAT AGT ACT TGG AAT AAT AAT ACT    1200
Tyr Cys Asn Ser Thr Gln Leu Phe Asn Ser Thr Trp Asn Asn Asn Thr
385                 390                 395                 400

GAA GGG TCA AAT AAC ACT GAA GGA AAT ACT ATC ACA CTC CCA TGC AGA    1248
Glu Gly Ser Asn Asn Thr Glu Gly Asn Thr Ile Thr Leu Pro Cys Arg
                405                 410                 415

ATA AAA CAA ATT ATA AAC ATG GTG CAG GAA GTA GGA AAA GCA ATG TAT    1296
Ile Lys Gln Ile Ile Asn Met Val Gln Glu Val Gly Lys Ala Met Tyr
            420                 425                 430

GCC CCT CCC ATC AGA GGA CAA ATT AGA TGT TCA TCA AAT ATT ACA GGG    1344
Ala Pro Pro Ile Arg Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr Gly
        435                 440                 445

CTG CTA TTA ACA AGA GAT GGT GGT ATT AAT GAG AAT GGG ACC GAG ATC    1392
Leu Leu Leu Thr Arg Asp Gly Gly Ile Asn Glu Asn Gly Thr Glu Ile
    450                 455                 460

TTC AGA CCT GGA GGA GGA GAT ATG AGG GAC AAT TGG AGA AGT GAA TTA    1440
Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu
465                 470                 475                 480

TAT AAA TAT AAA GTA GTA AAA ATT GAA CCA TTA GGA GTA GCA CCC ACC    1488
Tyr Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Val Ala Pro Thr
                485                 490                 495

AAG GCA AAG AGA AGA GTG GTG CAA AGA GAA AAA T GAGCGGCCGC           1532
Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys
            500                 505
```

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 507 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```
Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
  1               5                  10                  15

Ala Val Phe Val Ser Pro Ser Gln Glu Ile His Ala Arg Phe Arg Arg
             20                  25                  30

Gly Gly Arg Val Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
         35                  40                  45

Val Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
     50                  55                  60

Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
 65                  70                  75                  80

Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Glu Asn Val Thr Glu
                 85                  90                  95

His Phe Asn Met Trp Lys Asn Asn Met Val Glu Gln Met Gln Glu Asp
            100                 105                 110

Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
        115                 120                 125

Pro Leu Cys Val Thr Leu Asn Cys Lys Asp Val Asn Ala Thr Asn Thr
    130                 135                 140

Thr Asn Asp Ser Glu Gly Thr Met Glu Arg Gly Glu Ile Lys Asn Cys
145                 150                 155                 160

Ser Phe Asn Ile Thr Thr Ser Ile Arg Asp Glu Val Gln Lys Glu Tyr
                165                 170                 175

Ala Leu Phe Tyr Lys Leu Asp Val Val Pro Ile Asp Asn Asn Asn Thr
            180                 185                 190

Ser Tyr Arg Leu Ile Ser Cys Asp Thr Ser Val Ile Thr Gln Ala Cys
        195                 200                 205

Pro Lys Ile Ser Phe Glu Pro Ile Pro Ile His Tyr Cys Ala Pro Ala
    210                 215                 220

Gly Phe Ala Ile Leu Lys Cys Asn Asp Lys Thr Phe Asn Gly Lys Gly
225                 230                 235                 240

Pro Cys Lys Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro
                245                 250                 255

Val Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu
            260                 265                 270

Val Val Ile Arg Ser Asp Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile
        275                 280                 285

Val Gln Leu Lys Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn
    290                 295                 300

Asn Thr Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr
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305                 310                 315                 320

Thr Gly Glu Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser
                325                 330                 335

Arg Ala Lys Trp Asn Asp Thr Leu Lys Gln Ile Val Ile Lys Leu Arg
            340                 345                 350

Glu Gln Phe Glu Asn Lys Thr Ile Val Phe Asn His Ser Ser Gly Gly
        355                 360                 365

Asp Pro Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe
    370                 375                 380

Tyr Cys Asn Ser Thr Gln Leu Phe Asn Ser Thr Trp Asn Asn Asn Thr
385                 390                 395                 400

Glu Gly Ser Asn Asn Thr Glu Gly Asn Thr Ile Thr Leu Pro Cys Arg
                405                 410                 415

Ile Lys Gln Ile Ile Asn Met Val Gln Glu Val Gly Lys Ala Met Tyr
            420                 425                 430

Ala Pro Pro Ile Arg Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr Gly
        435                 440                 445

Leu Leu Leu Thr Arg Asp Gly Gly Ile Asn Glu Asn Gly Thr Glu Ile
    450                 455                 460

Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu
465                 470                 475                 480

Tyr Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Val Ala Pro Thr
                485                 490                 495

Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys
            500                 505
```

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

```
Ala Pro Thr Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys Arg
1               5                   10                  15
```

What is claimed is:

1. A recombinant nucleic acid molecule which encodes a mutant HIV-1 gp120 envelope glycoprotein comprising a V3 loop deletion and a C4 domain$_{(W->X)}$ point mutation, wherein X is an amino acid residue other than tryptophan.

2. The recombinant nucleic acid molecule of claim 1, wherein X is a valine residue.

3. The recombinant nucleic acid molecule of claim 1, wherein the nucleic acid molecule is a DNA molecule.

4. The recombinant nucleic acid molecule of claim 3, wherein the DNA molecule is a plasmid.

5. The recombinant nucleic acid molecule of claim 4, wherein the plasmid comprises the sequence of the plasmid designated PPI4-tPA.

6. The recombinant nucleic acid molecule of claim 1, wherein the C4 domain is an HIV-1_{LAI} gp120 envelope glycoprotein C4 domain.

7. The recombinant nucleic acid molecule of claim 6, wherein the mutant HIV-1 gp120 envelope glycoprotein is a mutant HIV-1_{LAI} gp120 envelope glycoprotein.

8. The recombinant nucleic acid molecule of claim 1, wherein the C4 domain is an HIV-1_{JR-FL} gp120 envelope glycoprotein C4 domain.

9. The recombinant nucleic acid molecule of claim 8,

wherein the mutant HIV-1 gp120 envelope glycoprotein is a mutant HIV-1_{JR-FL} gp120 envelope glycoprotein.

10. The mutant HIV-1 gp120 envelope glycoprotein encoded by the recombinant nucleic acid molecule of claim 1.

11. A vaccine which comprises a therapeutically effective amount of the mutant HIV-1 gp120 envelope glycoprotein of claim 10, and an adjuvant.

12. A method of treating an HIV-1-infected subject, which comprises immunizing the HIV-1-infected subject with the vaccine of claim 11, thereby treating the HIV-1-infected subject.

13. A vaccine which comprises a prophylactically effective amount of the mutant HIV-1 gp120 envelope glycoprotein of claim 10, and an adjuvant.

14. A method of reducing the likelihood of an HIV-1-exposed subject's becoming infected with HIV-1, which comprises immunizing the HIV-1-exposed subject with the vaccine of claim 13, thereby reducing the likelihood of the HIV-1-exposed subject's becoming infected with HIV-1.

15. A method of reducing the likelihood of a non-HIV-1-exposed subject's becoming infected with HIV-1, which comprises immunizing the non-HIV-1-exposed subject with the vaccine of claim 13, thereby reducing the likelihood of the non-HIV-1-exposed subject's becoming infected with HIV-1.

16. A method of obtaining partially purified antibodies which specifically bind to the CD4-binding domain of

HIV-1 gp120 envelope glycoprotein, which method comprises (a) immunizing a non-HIV-1-exposed subject with the vaccine of claim 13, (b) recovering from the immunized subject serum comprising said antibodies, and (c) partially purifying said antibodies, thereby obtaining partially purified antibodies which specifically bind to the CD4-binding domain of HIV-1 gp120 envelope glycoprotein.

17. The method of claim 16, wherein the subject is a human.

18. The partially purified antibodies produced by the method of claim 16.

19. A pharmaceutical composition, which comprises a therapeutically effective amount of the partially purified antibodies of claim 18, and a pharmaceutically acceptable carrier.

20. A method of treating an HIV-1-infected subject, which comprises administering to the subject a dose of the pharmaceutical composition of claim 19 effective to reduce the population of HIV-1-infected cells in the HIV-1-infected subject, thereby treating the HIV-1-infected subject.

21. A method of treating an HIV-1-infected subject, which comprises administering to the subject a dose of the pharmaceutical composition of claim 19 effective to reduce the population of HIV-1 in the HIV-1-infected subject, thereby treating the HIV-1-infected subject.

22. A composition which comprises a prophylactically effective amount of the partially purified antibodies of claim 18, and a pharmaceutically acceptable carrier.

23. A method of reducing the likelihood of an HIV-1-exposed subject's becoming infected with HIV-1, which comprises administering to the HIV-1-exposed subject a dose of the composition of claim 22 effective to reduce the population of HIV-1 in the HIV-1-exposed subject, thereby reducing the likelihood of the subject's becoming infected with HIV-1.

24. The method of claim 23, wherein the subject is a medical practitioner.

25. The method of claim 23, wherein the subject is a newborn infant.

26. A method of reducing the likelihood of a non-HIV-1-exposed subject's becoming infected with HIV-1 as a result of exposure thereto during an incident wherein there is an increased risk of exposure to HIV-1, which comprises administering to the subject immediately prior to the incident a dose of the composition of claim 22 effective to reduce the population of HIV-1 to which the subject is exposed during the incident, thereby reducing the likelihood of the subject's becoming infected with HIV-1.

27. The method of claim 26, wherein the subject is a medical practitioner.

FIGURE 1

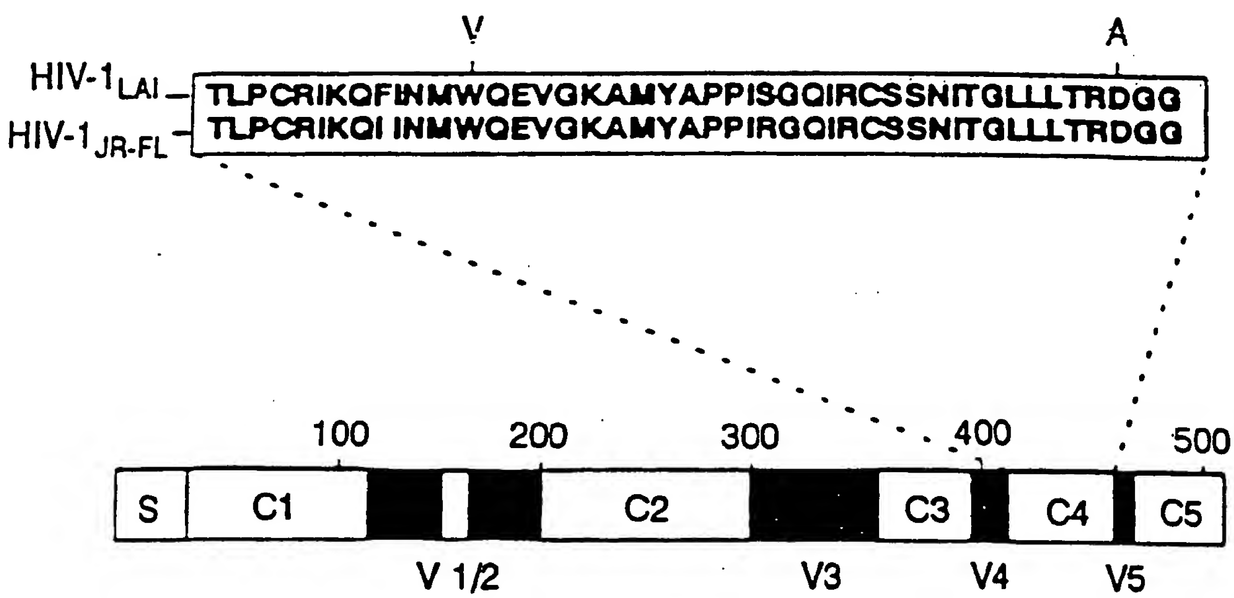

V
A
HIV-1LAI
TLPCRIKQFINMWQEVGKAMYAPPISGQIRCSSNITGLLLTRDGG
HIV-1JR-FL
TLPCRIKQI INMWQEVGKAMYAPPIRGQIRCSSNITGLLLTRDGG
100
200
300
400
500
S
C1
C2
C3
C4
C5
V 1/2
V3
V4
V5

FIGURE 2

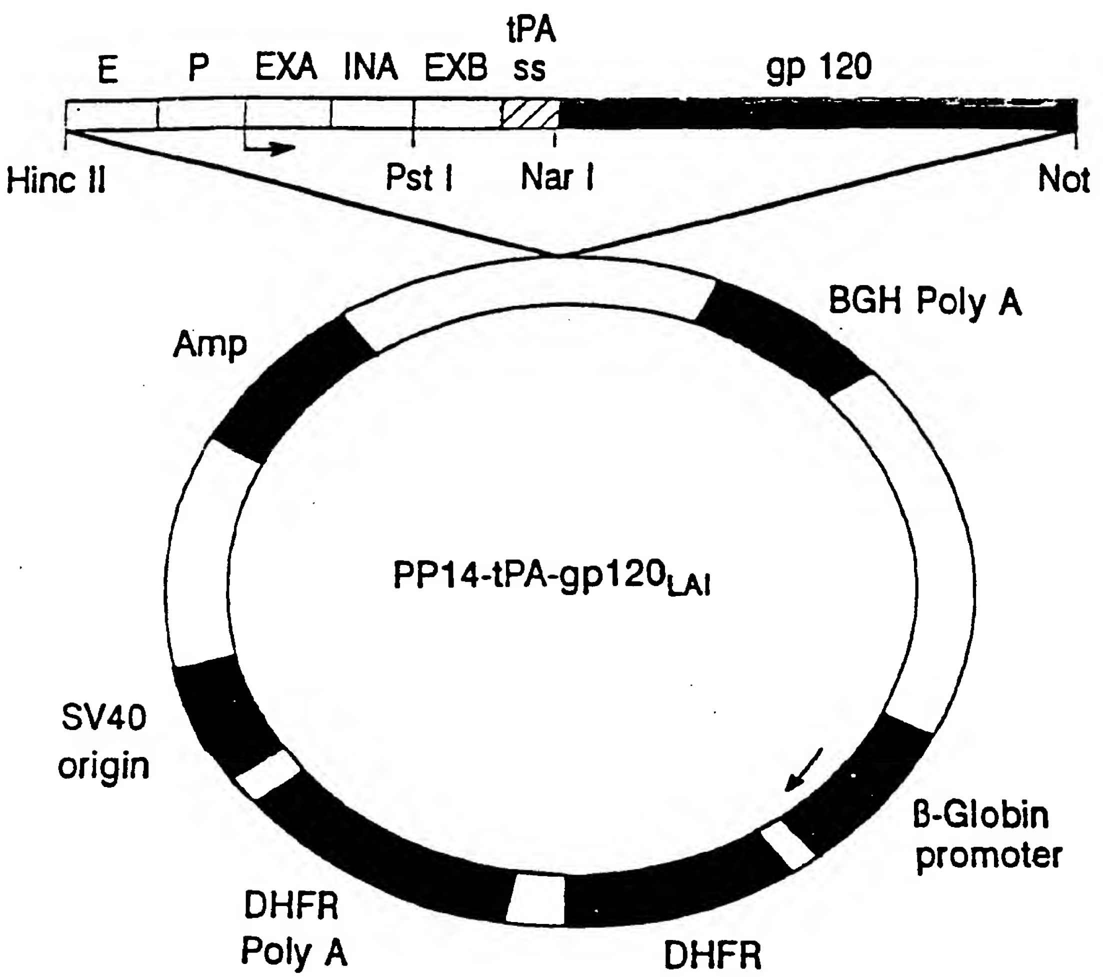

E
P
EXA
INA
EXB
tPA
ss
gp 120
Hinc II
Pst I
Nar I
Not I
BGH Poly A
Amp
PP14-tPA-gp120LAI
SV40
origin
ß-Globin
promoter
DHFR
Poly A
DHFR

FIGURE 3A
FIGURE 3B
FIGURE 3C
FIGURE 3D
FIGURE 3E
FIGURE 3F

FIGURE 3A

```
     HincII
1    ttgacattgattattgactagttattaatagtaatcaattacggggtcattagttcatagcccatatatgga
73   gttccgcgttacataacttacggtaaatggcccgcctggctgaccgcccaacgacccccgcccattgacgtc
145  aataatgacgtatgttcccatagtaacgccaatagggactttccattgacgtcaatgggtggactatttacg
217  gtaaactgcccacttggcagtacatcaagtgtatcatatgccaagtacgccccctattgacgtcaatgacgg
289  taaatggcccgcctggcattatgcccagtacatgaccttatgggactttcctacttggcagtacatctacgt
361  attagtcatcgctattaccatggtgatgcggttttggcagtacatcaatgggcgtggatagcggtttgactc
433  acggggatttccaagtctccaccccattgacgtcaatgggagtttgttttggcaccaaaatcaacgggactt
```

FIGURE 3B

```
505   tccaaaatgtcgtaacaactccgccccattgacgcaaatgggcggtaggcgtgtacggtgggaggtctatat
                                               Exon A
577   aagcagagctcgtttagtgaaccGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAG
                                   ➡ Transcription Start
649   ACACCGGGACCGATCCAGCCTCCGCGGCCGGGAACGGTGCATTGGAACGCGGATTCCCCGTGCCAAGAGTGA
                                              Intron A
721   Cgtaagtaccgcctatagactctataggcacacccctttggctcttatgcatgctatactgtttttggcttg

793   ggccaacaccccgtcctagataggtgatggtatagcttagcctataggtgtgggttattgaccattattgac

865   cactcccctattggtgacgatactttccattactaatccataacatggccgctctttgccacaactatctct

937   attggctatatgccaatactctgtccttcagagactgacacggactctgtatttttacaggatggggtccca

1009  tttattatttacaaattcacatatacaacaacgccgtcccccgtgcccgcagtttttattaacatgcggat

1081  ctccacgcgaatctcgggtacgtgttccggacatgggctcttctccggtagcggcggagctccacatccgag

1153  cctgtcccatgcccatgcctccagcggctcatggtcgctcggcagctccttgctcctaacagtggaggccag

1225  acttaggcacaggacaatgcccaccaccaccagtgtgccgcacaaggccgtggcggtagggtatgtgtctga
```

FIGURE 3C

```
1297  aaatgagctcggagattgggctcgcaccgctgacgcagatggaagacttaaggcagcggcagaagaagatgc

1369  aggcagctgagttgttgtattctgtagagttggaggtaactcccgttgcggtgctgttaacggtggagggca

1441  gtgtagtctgagcagtactcgttgctgccgcgcgcgccaccagacataatagctgacagactaacagactgt
                        PstI       Exon B                tPA signal sequence
1513  tcctttccatgggtctttctgcagTCACCGTCCTTGACACGATGGATGCAATGAAGAGAGGGCTCTGCTGT
   1                                                M  D  A  M  K  R  G  L  C  C
                                                                          NarI
1585  GTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCCAGCCAGGAAATCCATGCCCGATTCAGAAGAGGCGCC
  11   V  L  L  L  C  G  A  V  F  V  S  P  S  Q  E  I  H  A  R  F  R  R  G  A

1657  AGAACAGAAAAATTGTGGGTCACAGTCTATTATGGGGTACCTGTGTGGAAGGAAGCAACCACCACTCTATTT
  35   R  T  E  K  L  W  V  T  V  Y  Y  G  V  P  V  W  K  E  A  T  T  T  L  F
       ▲  Signal cleavage

1729  TGTGCATCAGATGCTAAAGCATATGATACAGAGGTACATAATGTTTGGGCCACACATGCCTGTGTACCCACA
  59   C  A  S  D  A  K  A  Y  D  T  E  V  H  N  V  W  A  T  H  A  C  V  P  T

1801  GACCCCAACCCACAAGAAGTAGTATTGGTAAATGTGACAGAAAATTTTAACATGTGGAAAAATGACATGGTA
  83   D  P  N  P  Q  E  V  V  L  V  N  V  T  E  N  F  N  M  W  K  N  D  M  V
```

FIGURE 3D

```
1873 GAACAGATGCATGAGGATATAATCAGTTTATGGGATCAAAGCCTAAAGCCATGTGTAAAATTAACCCCACTC
107   E  Q  M  H  E  D  I  I  S  L  W  D  Q  S  L  K  P  C  .  K  L  T  P  L

1945 TGTGTTAGTTTAAAGTGCACTGATTTGGGGAATGCTACTAATACCAATAGTAGTAATACCAATAGTAGTAGC
131   C  V  S  L  K  C  T  D  L  G  N  A  T  N  T  N  S  S  N  T  N  S  S  S

2017 GGGGAAATGATGATGGAGAAAGGAGAGATAAAAAACTGCTCTTTCAATATCAGCACAAGCATAAGAGGTAAG
155   G  E  M  M  M  E  K  G  E  I  K  N  C  S  F  N  I  S  T  S  I  R  G  K

2089 GTGCAGAAAGAATATGCATTTTTTTATAAACTTGATATAATACCAATAGATAATGATACTACCAGCTATACG
179   V  Q  K  E  Y  A  F  F  Y  K  L  D  I  I  P  I  D  N  D  T  T  S  Y  T

2161 TTGACAAGTTGTAACACCTCAGTCATTACACAGGCCTGTCCAAAGGTATCCTTTGAGCCAATTCCCATACAT
203   L  T  S  C  N  T  S  V  I  T  Q  A  C  P  K  V  S  F  E  P  I  P  I  H

2233 TATTGTGCCCCGGCTGGTTTTGCGATTCTAAAATGTAATAATAAGACGTTCAATGGAACAGGACCATGTACA
227   Y  C  A  P  A  G  F  A  I  L  K  C  N  N  K  T  F  N  G  T  G  P  C  T

2305 AATGTCAGCACAGTACAATGTACACATGGAATTAGGCCAGTAGTATCAACTCAACTGCTGTTGAATGGCAGT
251   N  V  S  T  V  Q  C  T  H  G  I  R  P  V  V  S  T  Q  L  L  L  N  G  S

2377 CTAGCAGAAGAAGAGGTAGTAATTAGATCTGCCAATTTCACAGACAATGCTAAAACCATAATAGTACAGCTG
275   L  A  E  E  E  V  V  I  R  S  A  N  F  T  D  N  A  K  T  I  I  V  Q  L
```

FIGURE 3E

```
2449  AACCAATCTGTAGAAATTAATTGTACAAGACCCAACAACAATACAAGAAAAAGTATCCGTATCCAGAGGGGA
 299   N  Q  S  V  E  I  N  C  T  R  P  N  N  N  T  R  K  S  I  R  I  Q  R  G

2521  CCAGGGAGAGCATTTGTTACAATAGGAAAAATAGGAAATATGAGACAAGCACATTGTAACATTAGTAGAGCA
 323   P  G  R  A  F  V  T  I  G  K  I  G  N  M  R  Q  A  H  C  N  I  S  R  A

2593  AAATGGAATGCCACTTTAAAACAGATAGCTAGCAAATTAAGAGAACAATTTGGAAATAATAAAACAATAATC
 347   K  W  N  A  T  L  K  Q  I  A  S  K  L  R  E  Q  F  G  N  N  K  T  I  I

2665  TTTAAGCAATCCTCAGGAGGGGACCCAGAAATTGTAACGCACAGTTTTAATTGTGGAGGGGAATTTTTCTAC
 371   F  K  Q  S  S  G  G  D  P  E  I  V  T  H  S  F  N  C  G  G  E  F  F  Y

2737  TGTAATTCAACACAACTGTTTAATAGTACTTGGTTTAATAGTACTTGGAGTACTGAAGGGTCAAATAACACT
 395   C  N  S  T  Q  L  F  N  S  T  W  F  N  S  T  W  S  T  E  G  S  N  N  T

2809  GAAGGAAGTGACACAATCACACTCCCATGCAGAATAAAACAATTTATAAACATGTGGCAGGAAGTAGGAAAA
 419   E  G  S  D  T  I  T  L  P  C  R  I  K  Q  F  I  N  M  W  Q  E  V  G  K

2881  GCAATGTATGCCCCTCCCATCAGCGGACAAATTAGATGTTCATCAAATATTACAGGGCTGCTATTAACAAGA
 443   A  M  Y  A  P  P  I  S  G  Q  I  R  C  S  S  N  I  T  G  L  L  L  T  R

2953  GATGGTGGTAATAACAACAATGGGTCCGAGATCTTCAGACCTGGAGGAGGAGATATGAGGGACAATTGGAGA
 467   D  G  G  N  N  N  N  G  S  E  I  F  R  P  G  G  G  D  M  R  D  N  W  R
```

FIGURE 3F

```
3025   AGTGAATTATATAAATATAAAGTAGTAAAAATTGAACCATTAGGAGTAGCACCCACCAAGGCAAAGAGAAGA
 491    S  E  L  Y  K  Y  K  V  V  K  I  E  P  L  G  V  A  P  T  K  A  K  R  R
                               NotI
3097   GTGGTGCAGAGAGAAAAATGAGCGGCCGC
 515    V  V  Q  R  E  K  -
```

FIGURE 4

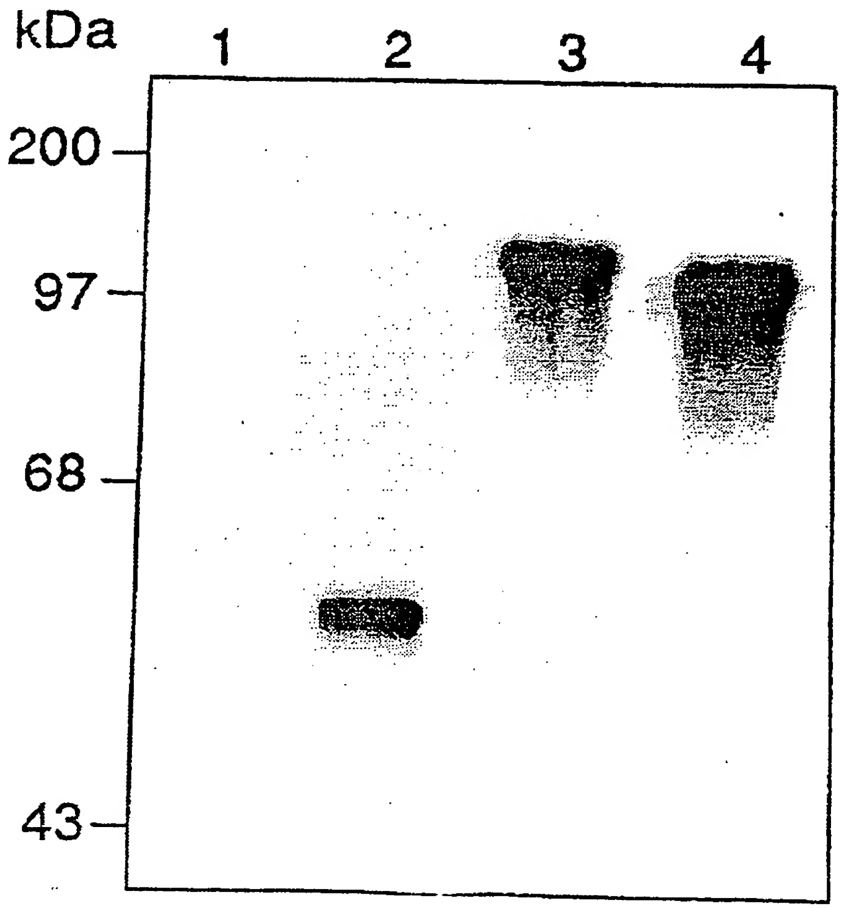


FIGURE 5A

Stable CHO clone	[gp120] (ng/ml)
5	6
6	14
9	123
10	4
12	18
13	18

FIGURE 5B

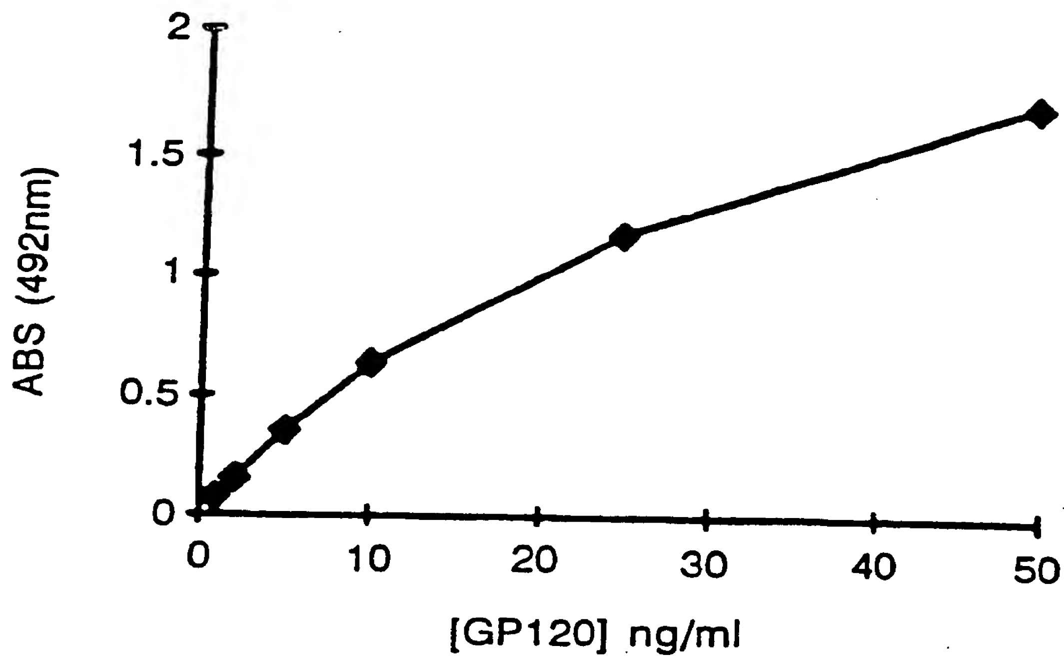


FIGURE 6

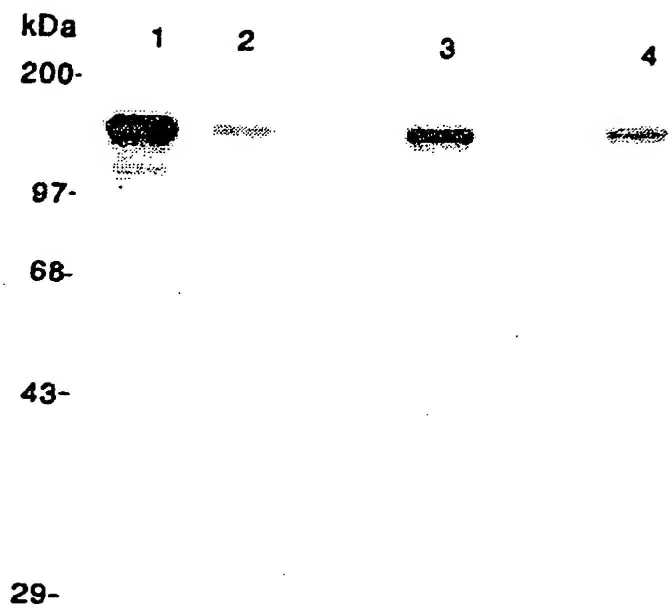


FIGURE 7A

FIGURE 7A
FIGURE 7B
FIGURE 7C

JR-FL

```
  1                                                                ATGGATGCAATGAAGAGA
  1                                                                 M  D  A  M  K  R

 19  GGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCCAGCCAGGAAATC
  7   G  L  C  C  V  L  L  L  C  G  A  V  F  V  S  P  S  Q  E  I
                         NarI
 79  CATGCCCGATTCAGAAGAGGCGGCAGAGTAGAAAAGTTGTGGGTCACAGTCTATTATGGG
 27   H  A  R  F  R  R  G  A  R  V  E  K  L  W  V  T  V  Y  Y  G
                             ▲Signal cleavage
139  GTACCTGTGTGGAAAGAAGCAACCACCACTCTATTTTGTGCATCAGATGCTAAAGCATAT
 47   V  P  V  W  K  E  A  T  T  T  L  F  C  A  S  D  A  K  A  Y

199  GATACAGAGGTACATAATGTTTGGGCCACACATGCCTGTGTACCCACAGACCCCAACCCA
 67   D  T  E  V  H  N  V  W  A  T  H  A  C  V  P  T  D  P  N  P

259  CAAGAAGTAGTATTGGAAAATGTAACAGAACATTTTAACATGTGGAAAAATAACATGGTA
 87   Q  E  V  V  L  E  N  V  T  E  H  F  N  M  W  K  N  N  M  V

319  GAACAGATGCAGGAGGATATAATCAGTTTATGGGATCAAAGCCTAAAGCCATGTGTAAAA
107   E  Q  M  Q  E  D  I  I  S  L  W  D  Q  S  L  K  P  C  V  K

379  TTAACCCCACTCTGTGTTACTTTAAATTGCAAGGATGTGAATGCTACTAATACCACTAAT
127   L  T  P  L  C  V  T  L  N  C  K  D  V  N  A  T  N  T  T  N

439  GATAGCGAGGGAACGATGGAGAGAGGAGAAATAAAAAACTGCTCTTTCAATATCACCACA
147   D  S  E  G  T  M  E  R  G  E  I  K  N  C  S  F  N  I  T  T
```

FIGURE 7B

```
499  AGCATAAGAGATGAGGTGCAGAAAGAATATGCTCTTTTTATAAACTTGATGTAGTACCA
167   S  I  R  D  E  V  Q  K  E  Y  A  L  F  Y  K  L  D  V  V  P

559  ATAGATAATAATAATACCAGCTATAGGTTGATAAGTTGTGACACCTCAGTCATTACACAG
187   I  D  N  N  N  T  S  Y  R  L  I  S  C  D  T  S  V  I  T  Q

619  GCCTGTCCAAAGATATCCTTTGAGCCAATTCCCATACATTATTGTGCCCCGGCTGGTTTT
207   A  C  P  K  I  S  F  E  P  I  P  I  H  Y  C  A  P  A  G  F

679  GCGATTCTAAAGTGTAATGATAAGACGTTCAATGGAAAAGGACCATGTAAAAATGTCAGC
227   A  I  L  K  C  N  D  K  T  F  N  G  K  G  P  C  K  N  V  S

739  ACAGTACAATGTACACATGGAATTAGGCCAGTAGTATCAACTCAACTGCTGCTAAATGGC
247   T  V  Q  C  T  H  G  I  R  P  V  V  S  T  Q  L  L  L  N  G

799  AGTCTAGCAGAAGAAGAGGTAGTAATTAGATCTGACAATTTCACGAACAATGCTAAAACC
267   S  L  A  E  E  E  V  V  I  R  S  D  N  F  T  N  N  A  K  T

859  ATAATAGTACAGCTGAAAGAATCTGTAGAAATTAATTGTACAAGACCCAACAACAATACA
287   I  I  V  Q  L  K  E  S  V  E  I  N  C  T  R  P  N  N  N  T

919  AGAAAAAGTATACATATAGGACCAGGGAGAGCATTTTATACTACAGGAGAAATAATAGGA
307   R  K  S  I  H  I  G  P  G  R  A  F  Y  T  T  G  E  I  I  G

979  GATATAAGACAAGCACATTGTAACATTAGTAGAGCAAAATGGAATGACACTTTAAAACAG
327   D  I  R  Q  A  H  C  N  I  S  R  A  K  W  N  D  T  L  K  Q
```

FIGURE 7C

```
1039   ATAGTTATAAAATTAAGAGAACAATTTGAGAATAAAACAATAGTCTTTAATCACTCCTCA
 347    I  V  I  K  L  R  E  Q  F  E  N  K  T  I  V  F  N  H  S  S

1099   GGAGGGGACCCAGAAATTGTAATGCACAGTTTTAATTGTGGAGGAGAATTTTTCTACTGT
 367    G  G  D  P  E  I  V  M  H  S  F  N  C  G  G  E  F  F  Y  C

1159   AATTCAACACAACTGTTTAATAGTACTTGGAATAATAATACTGAAGGGTCAAATAACACT
 387    N  S  T  Q  L  F  N  S  T  W  N  N  N  T  E  G  S  N  N  T

1219   GAAGGAAATACTATCACACTCCCATGCAGAATAAAACAAATTATAAACATGTGGCAGGAA
 407    E  G  N  T  I  T  L  P  C  R  I  K  Q  I  I  N  M  W  Q  E

1279   GTAGGAAAAGCAATGTATGCCCCTCCCATCAGAGGACAAATTAGATGTTCATCAAATATT
 427    V  G  K  A  M  Y  A  P  P  I  R  G  Q  I  R  C  S  S  N  I

1339   ACAGGGCTGCTATTAACAAGAGATGGTGGTATTAATGAGAATGGGACCGAGATCTTCAGA
 447    T  G  L  L  L  T  R  D  G  G  I  N  E  N  G  T  E  I  F  R

1399   CCTGGAGGAGGAGATATGAGGGACAATTGGAGAAGTGAATTATATAAATATAAAGTAGTA
 467    P  G  G  G  D  M  R  D  N  W  R  S  E  L  Y  K  Y  K  V  V

1459   AAAATTGAACCATTAGGAGTAGCACCCACCAAGGCAAAGAGAAGAGTGGTGCAAAGAGAA
 487    K  I  E  P  L  G  V  A  P  T  K  A  K  R  R  V  V  Q  R  E
                NotI
1519   AAATGAGCGGCCGC
 507    K
```

FIGURE 8A

FIGURE 8A
FIGURE 8B
FIGURE 8C

LAI ΔV3

```
  1                                      ATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTG
  1                                       M  D  A  M  K  R  G  L  C  C  V  L
                                                                  NarI
 37  CTGCTGTGTGGAGCAGTCTTCGTTTCGCCCAGCCAGGAAATCCATGCCCGATTCAGAAGAGGCGCCAGAACA
 13   L  L  C  G  A  V  F  V  S  P  S  Q  E  I  H  A  R  F  R  R  G  A  R  T
                                                      Signal cleavage ▲
109  GAAAAATTGTGGGTCACAGTCTATTATGGGGTACCTGTGTGGAAGGAAGCAACCACCACTCTATTTTGTGCA
 37   E  K  L  W  V  T  V  Y  Y  G  V  P  V  W  K  E  A  T  T  T  L  F  C  A

181  TCAGATGCTAAAGCATATGATACAGAGGTACATAATGTTTGGGCCACACATGCCTGTGTACCCACAGACCCC
 61   S  D  A  K  A  Y  D  T  E  V  H  N  V  W  A  T  H  A  C  V  P  T  D  P

253  AACCCACAAGAAGTAGTATTGGTAAATGTGACAGAAAATTTTAACATGTGGAAAAATGACATGGTAGAACAG
 85   N  P  Q  E  V  V  L  V  N  V  T  E  N  F  N  M  W  K  N  D  M  V  E  Q

325  ATGCATGAGGATATAATCAGTTTATGGGATCAAAGCCTAAAGCCATGTGTAAAATTAACCCCACTCTGTGTT
109   M  H  E  D  I  I  S  L  W  D  Q  S  L  K  P  C  V  K  L  T  P  L  C  V

397  AGTTTAAAGTGCACTGATTTGGGGAATGCTACTAATACCAATAGTAGTAATACCAATAGTAGTAGCGGGGAA
133   S  L  K  C  T  D  L  G  N  A  T  N  T  N  S  S  N  T  N  S  S  S  G  E
```

FIGURE 8B

```
469  ATGATGATGGAGAAAGGAGAGATAAAAAACTGCTCTTTCAATATCAGCACAAGCATAAGAGGTAAGGTGCAG
157   M  M  M  E  K  G  E  I  K  N  C  S  F  N  I  S  T  S  I  R  G  K  V  Q

541  AAAGAATATGCATTTTTTTATAAACTTGATATAATACCAATAGATAATGATACTACCAGCTATACGTTGACA
181   K  E  Y  A  F  F  Y  K  L  D  I  I  P  I  D  N  D  T  T  S  Y  T  L  T

613  AGTTGTAACACCTCAGTCATTACACAGGCCTGTCCAAAGGTATCCTTTGAGCCAATTCCCATACATTATTGT
205   S  C  N  T  S  V  I  T  Q  A  C  P  K  V  S  F  E  P  I  P  I  H  Y  C

685  GCCCCGGCTGGTTTTGCGATTCTAAAATGTAATAATAAGACGTTCAATGGAACAGGACCATGTACAAATGTC
229   A  P  A  G  F  A  I  L  K  C  N  N  K  T  F  N  G  T  G  P  C  T  N  V

757  AGCACAGTACAATGTACACATGGAATTAGGCCAGTAGTATCAACTCAACTGCTGTTGAATGGCAGTCTAGCA
253   S  T  V  Q  C  T  H  G  I  R  P  V  V  S  T  Q  L  L  L  N  G  S  L  A

829  GAAGAAGAGGTAGTAATTAGATCTGCCAATTTCACAGACAATGCTAAAACCATAATAGTACAGCTGAACCAA
277   E  E  E  V  V  I  R  S  A  N  F  T  D  N  A  K  T  I  I  V  Q  L  N  Q

901  TCTGTAGAAATTAATTGTACAGGTGCTGGACATTGTAACATTAGTAGAGCAAAATGGAATGCCACTTTAAAA
301   S  V  E  I  N  C  T  G  A  G  H  C  N  I  S  R  A  K  W  N  A  T  L  K
```

FIGURE 8C

```
973   CAGATAGCTAGCAAATTAAGAGAACAATTTGGAAATAATAAAACAATAATCTTTAAGCAATCCTCAGGAGGG
325    Q  I  A  S  K  L  R  E  Q  F  G  N  N  K  T  I  I  F  K  Q  S  S  G  G

1045  GACCCAGAAATTGTAACGCACAGTTTTAATTGTGGAGGGGAATTTTTCTACTGTAATTCAACACAACTGTTT
349    D  P  E  I  V  T  H  S  F  N  C  G  G  E  F  F  Y  C  N  S  T  Q  L  F

1117  AATAGTACTTGGTTTAATAGTACTTGGAGTACTGAAGGGTCAAATAACACTGAAGGAAGTGACACAATCACA
373    N  S  T  W  F  N  S  T  W  S  T  E  G  S  N  N  T  E  G  S  D  T  I  T

1189  CTCCCATGCAGAATAAAACAATTTATAAACATGTGGCAGGAAGTAGGAAAAGCAATGTATGCCCCTCCCATC
397    L  P  C  R  I  K  Q  F  I  N  M  W  Q  E  V  G  K  A  M  Y  A  P  P  I

1261  AGCGGACAAATTAGATGTTCATCAAATATTACAGGGCTGCTATTAACAAGAGATGGTGGTAATAACAACAAT
421    S  G  Q  I  R  C  S  S  N  I  T  G  L  L  L  T  R  D  G  G  N  N  N  N

1333  GGGTCCGAGATCTTCAGACCTGGAGGAGGAGATATGAGGGACAATTGGAGAAGTGAATTATATAAATATAAA
445    G  S  E  I  F  R  P  G  G  G  D  M  R  D  N  W  R  S  E  L  Y  K  Y  K

1405  GTAGTAAAAATTGAACCATTAGGAGTAGCACCCACCAAGGCAAAGAGAAGAGTGGTGCAGAGAGAAAAATGA
469    V  V  K  I  E  P  L  G  V  A  P  T  K  A  K  R  R  V  V  Q  R  E  K  -
        NotI
1477  GCGGCCGC
```

FIGURE 9A

FIGURE 9A
FIGURE 9B
FIGURE 9C

```
JR-FL ΔV3
  1                                                    ATGGATGCAATGAAGAGA
  1                                                    M  D  A  M  K  R

 19  GGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCCAGCCAGGAAATC
  7  G  L  C  C  V  L  L  L  C  G  A  V  F  V  S  P  S  Q  E  I
                         NarI
 79  CATGCCCGATTCAGAAGAGGCGGCAGAGTAGAAAAGTTGTGGGTCACAGTCTATTATGGG
 27  H  A  R  F  R  R  G  A  R  V  E  K  L  W  V  T  V  Y  Y  G
                                  ▲ Signal cleavage
139  GTACCTGTGTGGAAAGAAGCAACCACCACTCTATTTTGTGCATCAGATGCTAAAGCATAT
 47  V  P  V  W  K  E  A  T  T  T  L  F  C  A  S  D  A  K  A  Y

199  GATACAGAGGTACATAATGTTTGGGCCACACATGCCTGTGTACCCACAGACCCCAACCCA
 67  D  T  E  V  H  N  V  W  A  T  H  A  C  V  P  T  D  P  N  P

259  CAAGAAGTAGTATTGGAAAATGTAACAGAACATTTTAACATGTGGAAAAATAACATGGTA
 87  Q  E  V  V  L  E  N  V  T  E  H  F  N  M  W  K  N  N  M  V

319  GAACAGATGCAGGAGGATATAATCAGTTTATGGGATCAAAGCCTAAAGCCATGTGTAAAA
107  E  Q  M  Q  E  D  I  I  S  L  W  D  Q  S  L  K  P  C  V  K

379  TTAACCCCACTCTGTGTTACTTTAAATTGCAAGGATGTGAATGCTACTAATACCACTAAT
127  L  T  P  L  C  V  T  L  N  C  K  D  V  N  A  T  N  T  T  N
```

FIGURE 9B

```
439  GATAGCGAGGGAACGATGGAGAGAGGAGAAATAAAAAACTGCTCTTTCAATATCACCACA
147   D  S  E  G  T  M  E  R  G  E  I  K  N  C  S  F  N  I  T  T

499  AGCATAAGAGATGAGGTGCAGAAAGAATATGCTCTTTTTTATAAACTTGATGTAGTACCA
167   S  I  R  D  E  V  Q  K  E  Y  A  L  F  Y  K  L  D  V  V  P

559  ATAGATAATAATAATACCAGCTATAGGTTGATAAGTTGTGACACCTCAGTCATTACACAG
187   I  D  N  N  N  T  S  Y  R  L  I  S  C  D  T  S  V  I  T  Q

619  GCCTGTCCAAAGATATCCTTTGAGCCAATTCCCATACATTATTGTGCCCCGGCTGGTTTT
207   A  C  P  K  I  S  F  E  P  I  P  I  H  Y  C  A  P  A  G  F

679  GCGATTCTAAAGTGTAATGATAAGACGTTCAATGGAAAAGGACCATGTAAAAATGTCAGC
227   A  I  L  K  C  N  D  K  T  F  N  G  K  G  P  C  K  N  V  S

739  ACAGTACAATGTACACATGGAATTAGGCCAGTAGTATCAACTCAACTGCTGCTAAATGGC
247   T  V  Q  C  T  H  G  I  R  P  V  V  S  T  Q  L  L  L  N  G

799  AGTCTAGCAGAAGAAGAGGTAGTAATTAGATCTGACAATTTCACGAACAATGCTAAAACC
267   S  L  A  E  E  E  V  V  I  R  S  D  N  F  T  N  N  A  K  T

859  ATAATAGTACAGCTGAAAGAATCTGTAGAAATTAATTGTACAGGTGCTGGACATTGTAAC
287   I  I  V  Q  L  K  E  S  V  E  I  N  C  T  G  A  G  H  C  N

919  ATTAGTAGAGCAAAATGGAATGACACTTTAAAACAGATAGTTATAAAAATTAAGAGAACAA
307   I  S  R  A  K  W  N  D  T  L  K  Q  I  V  I  K  L  R  E  Q
```

FIGURE 9C

```
979   TTTGAGAATAAAACAATAGTCTTTAATCACTCCTCAGGAGGGGACCCAGAAATTGTAATG
327    F  E  N  K  T  I  V  F  N  H  S  S  G  G  D  P  E  I  V  M

1039  CACAGTTTTAATTGTGGAGGAGAATTTTTCTACTGTAATTCAACACAACTGTTTAATAGT
347    H  S  F  N  C  G  G  E  F  F  Y  C  N  S  T  Q  L  F  N  S

1099  ACTTGGAATAATAATACTGAAGGGTCAAATAACACTGAAGGAAATACTATCACACTCCCA
367    T  W  N  N  N  T  E  G  S  N  N  T  E  G  N  T  I  T  L  P

1159  TGCAGAATAAAACAAATTATAAACATGTGGCAGGAAGTAGGAAAAGCAATGTATGCCCCT
387    C  R  I  K  Q  I  I  N  M  W  Q  E  V  G  K  A  M  Y  A  P

1219  CCCATCAGAGGACAAATTAGATGTTCATCAAATATTACAGGGCTGCTATTAACAAGAGAT
407    P  I  R  G  Q  I  R  C  S  S  N  I  T  G  L  L  L  T  R  D

1279  GGTGGTATTAATGAGAATGGGACCGAGATCTTCAGACCTGGAGGAGGAGATATGAGGGAC
427    G  G  I  N  E  N  G  T  E  I  F  R  P  G  G  G  D  M  R  D

1339  AATTGGAGAAGTGAATTATATAAATATAAAGTAGTAAAAATTGAACCATTAGGAGTAGCA
447    N  W  R  S  E  L  Y  K  Y  K  V  V  K  I  E  P  L  G  V  A
                                                 NotI
1399  CCCACCAAGGCAAAGAGAAGAGTGGTGCAAAGAGAAAAATGAGCGGCCGC
487    P  T  K  A  K  R  R  V  V  Q  R  E  K  -
```

FIGURE 10A

FIGURE 10A
FIGURE 10B
FIGURE 10C

LAI ΔV3-CD4⁻

```
  1                                                ATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTG
  1                                                 M  D  A  M  K  R  G  L  C  C  V  L
                                                                                 NarI
 37 CTGCTGTGTGGAGCAGTCTTCGTTTCGCCCAGCCAGGAAATCCATGCCCGATTCAGAAGAGGCGCCAGAACA
 13  L  L  C  G  A  V  F  V  S  P  S  Q  E  I  H  A  R  F  R  R  G  A  R  T
                                                              Signal cleavage ▲
109 GAAAAATTGTGGGTCACAGTCTATTATGGGGTACCTGTGTGGAAGGAAGCAACCACCACTCTATTTTGTGCA
 37  E  K  L  W  V  T  V  Y  Y  G  V  P  V  W  K  E  A  T  T  T  L  F  C  A

181 TCAGATGCTAAAGCATATGATACAGAGGTACATAATGTTTGGGCCACACATGCCTGTGTACCCACAGACCCC
 61  S  D  A  K  A  Y  D  T  E  V  H  N  V  W  A  T  H  A  C  V  P  T  D  P

253 AACCCACAAGAAGTAGTATTGGTAAATGTGACAGAAAATTTTAACATGTGGAAAAATGACATGGTAGAACAG
 85  N  P  Q  E  V  V  L  V  N  V  T  E  N  F  N  M  W  K  N  D  M  V  E  Q

325 ATGCATGAGGATATAATCAGTTTATGGGATCAAAGCCTAAAGCCATGTGTAAAATTAACCCCACTCTGTGTT
109  M  H  E  D  I  I  S  L  W  D  Q  S  L  K  P  C  V  K  L  T  P  L  C  V

397 AGTTTAAAGTGCACTGATTTGGGGAATGCTACTAATACCAATAGTAGTAATACCAATAGTAGTAGCGGGGAA
133  S  L  K  C  T  D  L  G  N  A  T  N  T  N  S  S  N  T  N  S  S  S  G  E
```

FIGURE 10B

```
469  ATGATGATGGAGAAAGGAGAGATAAAAAACTGCTCTTTCAATATCAGCACAAGCATAAGAGGTAAGGTGCAG
157   M  M  M  E  K  G  E  I  K  N  C  S  F  N  I  S  T  S  I  R  G  K  V  Q

541  AAAGAATATGCATTTTTTTATAAACTTGATATAATACCAATAGATAATGATACTACCAGCTATACGTTGACA
181   K  E  Y  A  F  F  Y  K  L  D  I  I  P  I  D  N  D  T  T  S  Y  T  L  T

613  AGTTGTAACACCTCAGTCATTACACAGGCCTGTCCAAAGGTATCCTTTGAGCCAATTCCCATACATTATTGT
205   S  C  N  T  S  V  I  T  Q  A  C  P  K  V  S  F  E  P  I  P  I  H  Y  C

685  GCCCCGGCTGGTTTTGCGATTCTAAAATGTAATAATAAGACGTTCAATGGAACAGGACCATGTACAAATGTC
229   A  P  A  G  F  A  I  L  K  C  N  N  K  T  F  N  G  T  G  P  C  T  N  V

757  AGCACAGTACAATGTACACATGGAATTAGGCCAGTAGTATCAACTCAACTGCTGTTGAATGGCAGTCTAGCA
253   S  T  V  Q  C  T  H  G  I  R  P  V  V  S  T  Q  L  L  L  N  G  S  L  A

829  GAAGAAGAGGTAGTAATTAGATCTGCCAATTTCACAGACAATGCTAAAACCATAATAGTACAGCTGAACCAA
277   E  E  E  V  V  I  R  S  A  N  F  T  D  N  A  K  T  I  I  V  Q  L  N  Q

901  TCTGTAGAAATTAATTGTACAGGTGCTGGACATTGTAACATTAGTAGAGCAAAATGGAATGCCACTTTAAAA
301   S  V  E  I  N  C  T  G  A  G  H  C  N  I  S  R  A  K  W  N  A  T  L  K

973  CAGATAGCTAGCAAATTAAGAGAACAATTTGGAAATAATAAAACAATAATCTTTAAGCAATCCTCAGGAGGG
325   Q  I  A  S  K  L  R  E  Q  F  G  N  N  K  T  I  I  F  K  Q  S  S  G  G
```

FIGURE 10C

```
1045  GACCCAGAAATTGTAACGCACAGTTTTAATTGTGGAGGGGAATTTTTCTACTGTAATTCAACACAACTGTTT
 349   D  P  E  I  V  T  H  S  F  N  C  G  G  E  F  F  Y  C  N  S  T  Q  L  F

1117  AATAGTACTTGGTTTAATAGTACTTGGAGTACTGAAGGGTCAAATAACACTGAAGGAAGTGACACAATCACA
 373   N  S  T  W  F  N  S  T  W  S  T  E  G  S  N  N  T  E  G  S  D  T  I  T

1189  CTCCCATGCAGAATAAAACAATTTATAAACATGGTGCAGGAAGTAGGAAAAGCAATGTATGCCCCTCCCATC
 397   L  P  C  R  I  K  Q  F  I  N  M  V  Q  E  V  G  K  A  M  Y  A  P  P  I

1261  AGCGGACAAATTAGATGTTCATCAAATATTACAGGGCTGCTATTAACAAGAGATGGTGGTAATAACAACAAT
 421   S  G  Q  I  R  C  S  S  N  I  T  G  L  L  L  T  R  D  G  G  N  N  N  N

1333  GGGTCCGAGATCTTCAGACCTGGAGGAGGAGATATGAGGGACAATTGGAGAAGTGAATTATATAAATATAAA
 445   G  S  E  I  F  R  P  G  G  G  D  M  R  D  N  W  R  S  E  L  Y  K  Y  K

1405  GTAGTAAAAATTGAACCATTAGGAGTAGCACCCACCAAGGCAAAGAGAAGAGTGGTGCAGAGAGAAAAATGA
 469   V  V  K  I  E  P  L  G  V  A  P  T  K  A  K  R  R  V  V  Q  R  E  K  -
        NotI
1447  GCGGCCGC
```

FIGURE 11A

FIGURE 11A
FIGURE 11B
FIGURE 11C

```
JR-FL ΔV3-CD4⁻
  1                                                            ATGGATGCAATGAAGAGA
  1                                                            M  D  A  M  K  R

 19   GGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCCAGCCAGGAAATC
  7   G  L  C  C  V  L  L  L  C  G  A  V  F  V  S  P  S  Q  E  I
                    NarI
 79   CATGCCCGATTCAGAAGAGGCGGCAGAGTAGAAAAGTTGTGGGTCACAGTCTATTATGGG
 27   H  A  R  F  R  R  G  A  R  V  E  K  L  W  V  T  V  Y  Y  G
                                ▲ Signal cleavage
139   GTACCTGTGTGGAAAGAAGCAACCACCACTCTATTTTGTGCATCAGATGCTAAAGCATAT
 47   V  P  V  W  K  E  A  T  T  T  L  F  C  A  S  D  A  K  A  Y

199   GATACAGAGGTACATAATGTTTGGGCCACACATGCCTGTGTACCCACAGACCCCAACCCA
 67   D  T  E  V  H  N  V  W  A  T  H  A  C  V  P  T  D  P  N  P

259   CAAGAAGTAGTATTGGAAAATGTAACAGAACATTTTAACATGTGGAAAAATAACATGGTA
 87   Q  E  V  V  L  E  N  V  T  E  H  F  N  M  W  K  N  N  M  V

319   GAACAGATGCAGGAGGATATAATCAGTTTATGGGATCAAAGCCTAAAGCCATGTGTAAAA
107   E  Q  M  Q  E  D  I  I  S  L  W  D  Q  S  L  K  P  C  V  K

379   TTAACCCCACTCTGTGTTACTTTAAATTGCAAGGATGTGAATGCTACTAATACCACTAAT
127   L  T  P  L  C  V  T  L  N  C  K  D  V  N  A  T  N  T  T  N
```

FIGURE 11B

```
439  GATAGCGAGGGAACGATGGAGAGAGGAGAAATAAAAAACTGCTCTTTCAATATCACCACA
147   D  S  E  G  T  M  E  R  G  E  I  K  N  C  S  F  N  I  T  T

499  AGCATAAGAGATGAGGTGCAGAAAGAATATGCTCTTTTTTATAAACTTGATGTAGTACCA
167   S  I  R  D  E  V  Q  K  E  Y  A  L  F  Y  K  L  D  V  V  P

559  ATAGATAATAATAATACCAGCTATAGGTTGATAAGTTGTGACACCTCAGTCATTACACAG
187   I  D  N  N  N  T  S  Y  R  L  I  S  C  D  T  S  V  I  T  Q

619  GCCTGTCCAAAGATATCCTTTGAGCCAATTCCCATACATTATTGTGCCCCGGCTGGTTTT
207   A  C  P  K  I  S  F  E  P  I  P  I  H  Y  C  A  P  A  G  F

679  GCGATTCTAAAGTGTAATGATAAGACGTTCAATGGAAAAGGACCATGTAAAAATGTCAGC
227   A  I  L  K  C  N  D  K  T  F  N  G  K  G  P  C  K  N  V  S

739  ACAGTACAATGTACACATGGAATTAGGCCAGTAGTATCAACTCAACTGCTGCTAAATGGC
247   T  V  Q  C  T  H  G  I  R  P  V  V  S  T  Q  L  L  L  N  G

799  AGTCTAGCAGAAGAAGAGGTAGTAATTAGATCTGACAATTTCACGAACAATGCTAAAACC
267   S  L  A  E  E  E  V  V  I  R  S  D  N  F  T  N  N  A  K  T

859  ATAATAGTACAGCTGAAAGAATCTGTAGAAATTAATTGTACAGGTGCTGGACATTGTAAC
287   I  I  V  Q  L  K  E  S  V  E  I  N  C  T  G  A  G  H  C  N

919  ATTAGTAGAGCAAAATGGAATGACACTTTAAAACAGATAGTTATAAAATTAAGAGAACAA
307   I  S  R  A  K  W  N  D  T  L  K  Q  I  V  I  K  L  R  E  Q
```

FIGURE 11C

```
979   TTTGAGAATAAAACAATAGTCTTTAATCACTCCTCAGGAGGGGACCCAGAAATTGTAATG
327    F  E  N  K  T  I  V  F  N  H  S  S  G  G  D  P  E  I  V  M

1039  CACAGTTTTAATTGTGGAGGAGAATTTTTCTACTGTAATTCAACACAACTGTTTAATAGT
347    H  S  F  N  C  G  G  E  F  F  Y  C  N  S  T  Q  L  F  N  S

1099  ACTTGGAATAATAATACTGAAGGGTCAAATAACACTGAAGGAAATACTATCACACTCCCA
367    T  W  N  N  N  T  E  G  S  N  N  T  E  G  N  T  I  T  L  P

1159  TGCAGAATAAAACAAATTATAAACATGGTGCAGGAAGTAGGAAAAGCAATGTATGCCCCT
387    C  R  I  K  Q  I  I  N  M  V  Q  E  V  G  K  A  M  Y  A  P

1219  CCCATCAGAGGACAAATTAGATGTTCATCAAATATTACAGGGCTGCTATTAACAAGAGAT
407    P  I  R  G  Q  I  R  C  S  S  N  I  T  G  L  L  L  T  R  D

1279  GGTGGTATTAATGAGAATGGGACCGAGATCTTCAGACCTGGAGGAGGAGATATGAGGGAC
427    G  G  I  N  E  N  G  T  E  I  F  R  P  G  G  G  D  M  R  D

1339  AATTGGAGAAGTGAATTATATAAATATAAAGTAGTAAAAATTGAACCATTAGGAGTAGCA
447    N  W  R  S  E  L  Y  K  Y  K  V  V  K  I  E  P  L  G  V  A
                                                    NotI
1399  CCCACCAAGGCAAAGAGAAGAGTGGTGCAAAGAGAAAAATGAGCGGCCGC
487    P  T  K  A  K  R  R  V  V  Q  R  E  K  -
```

FIGURE 12A

FIGURE 12A
FIGURE 12B
FIGURE 12C

LAI CD4⁻

```
                                                   tPA signal sequence
  1                                             ATGGATGCAATGAAGAGAGGGCTCTGCTGT
  1                                             M  D  A  M  K  R  G  L  C  C
                                                                          NarI
 37  GTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCCAGCCAGGAAATCCATGCCCGATTCAGAAGAGGCGCC
 13  V  L  L  L  C  G  A  V  F  V  S  P  S  Q  E  I  H  A  R  F  R  R  G  A

109  AGAACAGAAAAATTGTGGGTCACAGTCTATTATGGGGTACCTGTGTGGAAGGAAGCAACCACCACTCTATTT
 37  R  T  E  K  L  W  V  T  V  Y  Y  G  V  P  V  W  K  E  A  T  T  T  L  F
     ▲ Signal cleavage
181  TGTGCATCAGATGCTAAAGCATATGATACAGAGGTACATAATGTTTGGGCCACACATGCCTGTGTACCCACA
 61  C  A  S  D  A  K  A  Y  D  T  E  V  H  N  V  W  A  T  H  A  C  V  P  T

253  GACCCCAACCCACAAGAAGTAGTATTGGTAAATGTGACAGAAAATTTTAACATGTGGAAAAATGACATGGTA
 85  D  P  N  P  Q  E  V  V  L  V  N  V  T  E  N  F  N  M  W  K  N  D  M  V

325  GAACAGATGCATGAGGATATAATCAGTTTATGGGATCAAAGCCTAAAGCCATGTGTAAAATTAACCCCACTC
109  E  Q  M  H  E  D  I  I  S  L  W  D  Q  S  L  K  P  C  V  K  L  T  P  L

397  TGTGTTAGTTTAAAGTGCACTGATTTGGGGAATGCTACTAATACCAATAGTAGTAATACCAATAGTAGTAGC
133  C  V  S  L  K  C  T  D  L  G  N  A  T  N  T  N  S  S  N  T  N  S  S  S
```

FIGURE 12B

469 GGGGAAATGATGATGGAGAAAGGAGAGATAAAAAACTGCTCTTTCAATATCAGCACAAGCATAAGAGGTAAG
157 G E M M M E K G E I K N C S F N I S T S I R G K

541 GTGCAGAAAGAATATGCATTTTTTTATAAACTTGATATAATACCAATAGATAATGATACTACCAGCTATACG
181 V Q K E Y A F F Y K L D I I P I D N D T T S Y T

613 TTGACAAGTTGTAACACCTCAGTCATTACACAGGCCTGTCCAAAGGTATCCTTTGAGCCAATTCCCATACAT
205 L T S C N T S V I T Q A C P K V S F E P I P I H

685 TATTGTGCCCCGGCTGGTTTTGCGATTCTAAAATGTAATAATAAGACGTTCAATGGAACAGGACCATGTACA
229 Y C A P A G F A I L K C N N K T F N G T G P C T

757 AATGTCAGCACAGTACAATGTACACATGGAATTAGGCCAGTAGTATCAACTCAACTGCTGTTGAATGGCAGT
253 N V S T V Q C T H G I R P V V S T Q L L L N G S

829 CTAGCAGAAGAAGAGGTAGTAATTAGATCTGCCAATTTCACAGACAATGCTAAAACCATAATAGTACAGCTG
277 L A E E E V V I R S A N F T D N A K T I I V Q L

901 AACCAATCTGTAGAAATTAATTGTACAAGACCCAACAACAATACAAGAAAAAGTATCCGTATCCAGAGGGGA
301 N Q S V E I N C T R P N N N T R K S I R I Q R G

973 CCAGGGAGAGCATTTGTTACAATAGGAAAAATAGGAAATATGAGACAAGCACATTGTAACATTAGTAGAGCA
325 P G R A F V T I G K I G N M R Q A H C N I S R A

FIGURE 12C

```
1045  AAATGGAATGCCACTTTAAAACAGATAGCTAGCAAATTAAGAGAACAATTTGGAAATAATAAAACAATAATC
 349   K  W  N  A  T  L  K  Q  I  A  S  K  L  R  E  Q  F  G  N  N  K  T  I  I

1117  TTTAAGCAATCCTCAGGAGGGGACCCAGAAATTGTAACGCACAGTTTTAATTGTGGAGGGGAATTTTTCTAC
 373   F  K  Q  S  S  G  G  D  P  E  I  V  T  H  S  F  N  C  G  G  E  F  F  Y

1189  TGTAATTCAACACAACTGTTTAATAGTACTTGGTTTAATAGTACTTGGAGTACTGAAGGGTCAAATAACACT
 397   C  N  S  T  Q  L  F  N  S  T  W  F  N  S  T  W  S  T  E  G  S  N  N  T

1261  GAAGGAAGTGACACAATCACACTCCCATGCAGAATAAAACAATTTATAAACATGGTGAGGAAGTAGGAAAA
 421   E  G  S  D  T  I  T  L  P  C  R  I  K  Q  F  I  N  M  V  Q  E  V  G  K

1333  GCAATGTATGCCCCTCCCATCAGCGGACAAATTAGATGTTCATCAAATATTACAGGGCTGCTATTAACAAGA
 445   A  M  Y  A  P  P  I  S  G  Q  I  R  C  S  S  N  I  T  G  L  L  L  T  R

1405  GATGGTGGTAATAACAACAATGGGTCCGAGATCTTCAGACCTGGAGGAGGAGATATGAGGGACAATTGGAGA
 469   D  G  G  N  N  N  N  G  S  E  I  F  R  P  G  G  G  D  M  R  D  N  W  R

1477  AGTGAATTATATAAATATAAAGTAGTAAAAATTGAACCATTAGGAGTAGCACCCACCAAGGCAAAGAGAAGA
 493   S  E  L  Y  K  Y  K  V  V  K  I  E  P  L  G  V  A  P  T  K  A  K  R  R
                                 NotI
1549  GTGGTGCAGAGAGAAAAATGAGCGGCCGC
 517   V  V  Q  R  E  K  -
```

FIGURE 13A

FIGURE 13A
FIGURE 13B
FIGURE 13C
FIGURE 13D

JR-FL CD4⁻

```
  1                                                     ATGGATGCAATGAAGAGA
  1                                                      M  D  A  M  K  R

 19  GGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCCAGCCAGGAAATC
  7   G  L  C  C  V  L  L  L  C  G  A  V  F  V  S  P  S  Q  E  I
                         NarI
 79  CATGCCCGATTCAGAAGAGGCGGCAGAGTAGAAAAGTTGTGGGTCACAGTCTATTATGGG
 27   H  A  R  F  R  R  G  A  R  V  E  K  L  W  V  T  V  Y  Y  G
                              ▲ Signal cleavage
139  GTACCTGTGTGGAAAGAAGCAACCACCACTCTATTTTGTGCATCAGATGCTAAAGCATAT
 47   V  P  V  W  K  E  A  T  T  T  L  F  C  A  S  D  A  K  A  Y

199  GATACAGAGGTACATAATGTTTGGGCCACACATGCCTGTGTACCCACAGACCCCAACCCA
 67   D  T  E  V  H  N  V  W  A  T  H  A  C  V  P  T  D  P  N  P

259  CAAGAAGTAGTATTGGAAAATGTAACAGAACATTTTAACATGTGGAAAAATAACATGGTA
 87   Q  E  V  V  L  E  N  V  T  E  H  F  N  M  W  K  N  N  M  V
```

FIGURE 13B

```
319  GAACAGATGCAGGAGGATATAATCAGTTTATGGGATCAAAGCCTAAAGCCATGTGTAAAA
107   E  Q  M  Q  E  D  I  I  S  L  W  D  Q  S  L  K  P  C  V  K

379  TTAACCCCACTCTGTGTTACTTTAAATTGCAAGGATGTGAATGCTACTAATACCACTAAT
127   L  T  P  L  C  V  T  L  N  C  K  D  V  N  A  T  N  T  T  N

439  GATAGCGAGGGAACGATGGAGAGAGGAGAAATAAAAAACTGCTCTTTCAATATCACCACA
147   D  S  E  G  T  M  E  R  G  E  I  K  N  C  S  F  N  I  T  T

499  AGCATAAGAGATGAGGTGCAGAAAGAATATGCTCTTTTTTATAAACTTGATGTAGTACCA
167   S  I  R  D  E  V  Q  K  E  Y  A  L  F  Y  K  L  D  V  V  P

559  ATAGATAATAATAATACCAGCTATAGGTTGATAAGTTGTGACACCTCAGTCATTACACAG
187   I  D  N  N  N  T  S  Y  R  L  I  S  C  D  T  S  V  I  T  Q

619  GCCTGTCCAAAGATATCCTTTGAGCCAATTCCCATACATTATTGTGCCCCGGCTGGTTTT
207   A  C  P  K  I  S  F  E  P  I  P  I  H  Y  C  A  P  A  G  F
```

FIGURE 13C

```
679  GCGATTCTAAAGTGTAATGATAAGACGTTCAATGGAAAAGGACCATGTAAAAATGTCAGC
227   A  I  L  K  C  N  D  K  T  F  N  G  K  G  P  C  K  N  V  S

739  ACAGTACAATGTACACATGGAATTAGGCCAGTAGTATCAACTCAACTGCTGCTAAATGGC
247   T  V  Q  C  T  H  G  I  R  P  V  V  S  T  Q  L  L  L  N  G

799  AGTCTAGCAGAAGAAGAGGTAGTAATTAGATCTGACAATTTCACGAACAATGCTAAAACC
267   S  L  A  E  E  E  V  V  I  R  S  D  N  F  T  N  N  A  K  T

859  ATAATAGTACAGCTGAAAGAATCTGTAGAAATTAATTGTACAAGACCCAACAACAATACA
287   I  I  V  Q  L  K  E  S  V  E  I  N  C  T  R  F  N  N  N  T

919  AGAAAAAGTATACATATAGGACCAGGGAGAGCATTTTATACTACAGGAGAAATAATAGGA
307   R  K  S  I  H  I  G  P  G  R  A  F  Y  T  T  G  E  I  I  G

979  GATATAAGACAAGCACATTGTAACATTAGTAGAGCAAAATGGAATGACACTTTAAAACAG
327   D  I  R  Q  A  H  C  N  I  S  R  A  K  W  N  D  T  L  K  Q
```

FIGURE 13D

```
1039  ATAGTTATAAAATTAAGAGAACAATTTGAGAATAAAACAATAGTCTTTAATCACTCCTCA
 347   I  V  I  K  L  R  E  Q  F  E  N  K  T  I  V  F  N  H  S  S

1099  GGAGGGGACCCAGAAATTGTAATGCACAGTTTTAATTGTGGAGGAGAATTTTTCTACTGT
 367   G  G  D  P  E  I  V  M  H  S  F  N  C  G  G  E  F  F  Y  C

1159  AATTCAACACAACTGTTTAATAGTACTTGGAATAATAATACTGAAGGGTCAAATAACACT
 387   N  S  T  Q  L  F  N  S  T  W  N  N  N  T  E  G  S  N  N  T

1219  GAAGGAAATACTATCACACTCCCATGCAGAATAAAACAAATTATAAACATGGTGCAGGAA
 407   E  G  N  T  I  T  L  P  C  R  I  K  Q  I  I  N  M  V  Q  E

1279  GTAGGAAAAGCAATGTATGCCCCTCCCATCAGAGGACAAATTAGATGTTCATCAAATATT
 427   V  G  K  A  M  Y  A  P  P  I  R  G  Q  I  R  C  S  S  N  I

1339  ACAGGGCTGCTATTAACAAGAGATGGTGGTATTAATGAGAATGGGACCGAGATCTTCAGA
 447   T  G  L  L  L  T  R  D  G  G  I  N  E  N  G  T  E  I  F  R

1399  CCTGGAGGAGGAGATATGAGGGACAATTGGAGAAGTGAATTATATAAATATAAAGTAGTA
 467   P  G  G  G  D  M  R  D  N  W  R  S  E  L  Y  K  Y  K  V  V

1459  AAAATTGAACCATTAGGAGTAGCACCCACCAAGGCAAAGAGAAGAGTGGTGCAAAGAGAA
 487   K  I  E  P  L  G  V  A  P  T  K  A  K  R  R  V  V  Q  R  E
                NotI
1519  AAATGAGCGGCCGC
 507   K
```

FIGURE 14A

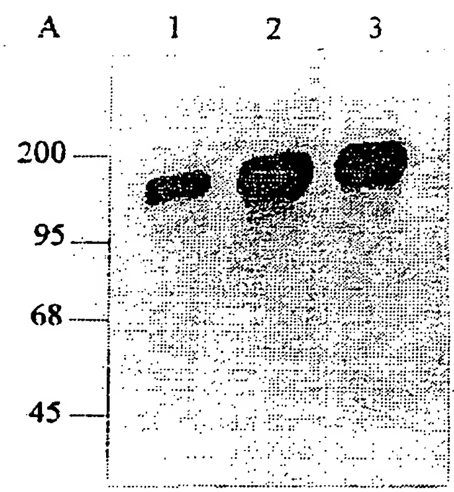


FIGURE 14B

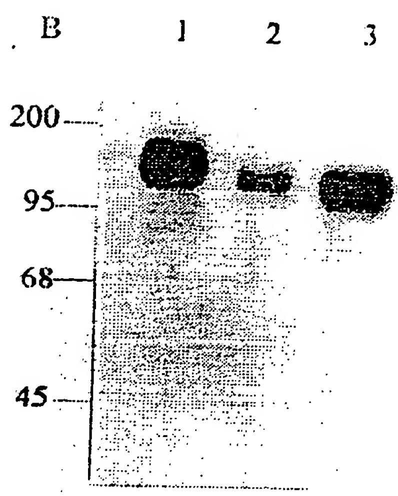


FIGURE 15A

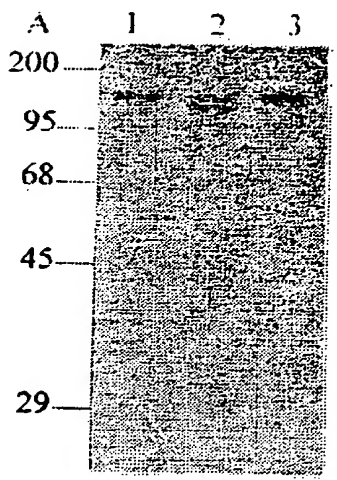


FIGURE 15B

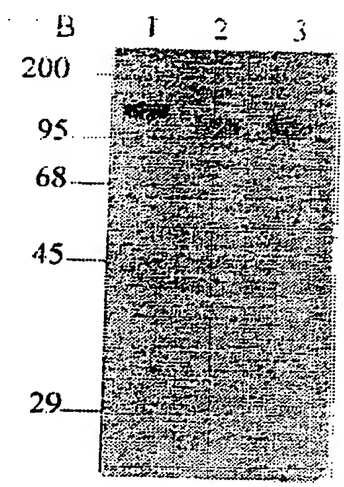
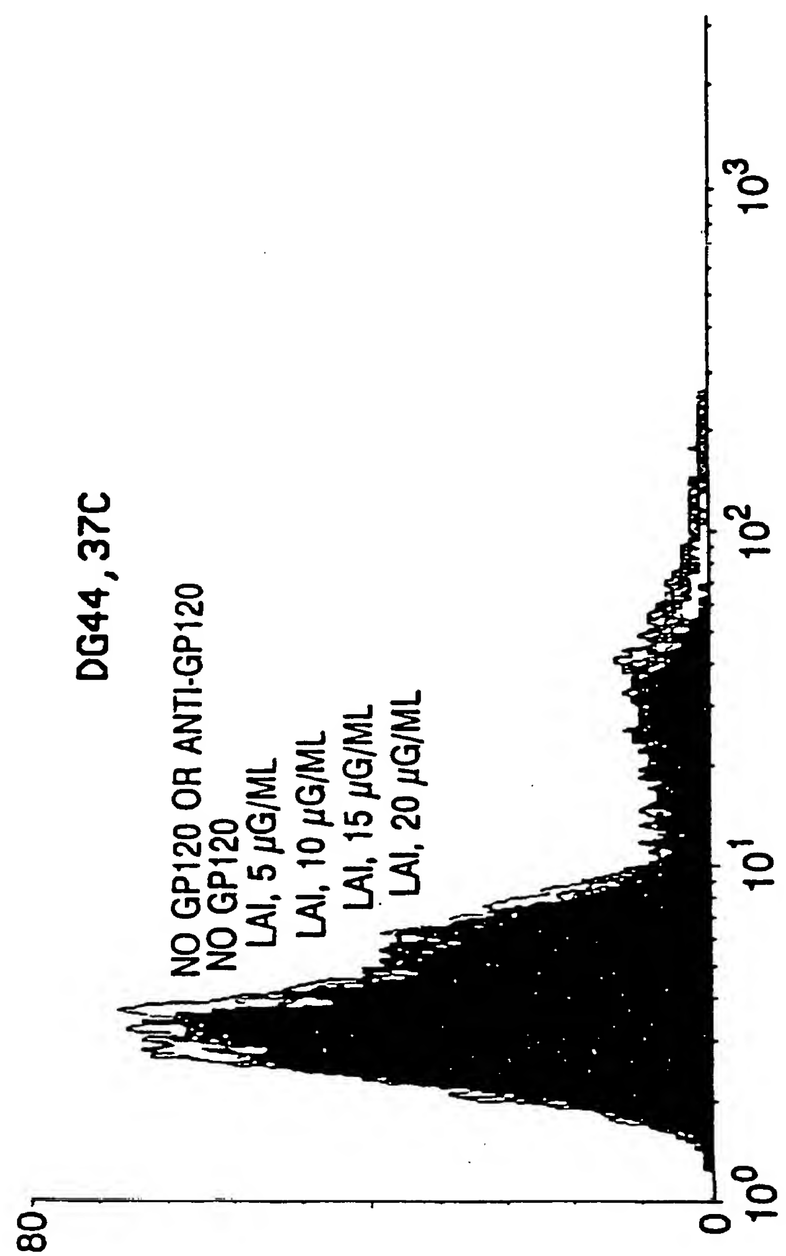


FIGURE 16A


DG44, 37C
NO GP120 OR ANTI-GP120
NO GP120
LAI, 5 µG/ML
LAI, 10 µG/ML
LAI, 15 µG/ML
LAI, 20 µG/ML
80
0
10^0
10^1
10^2
10^3

40/42

FIGURE 16B

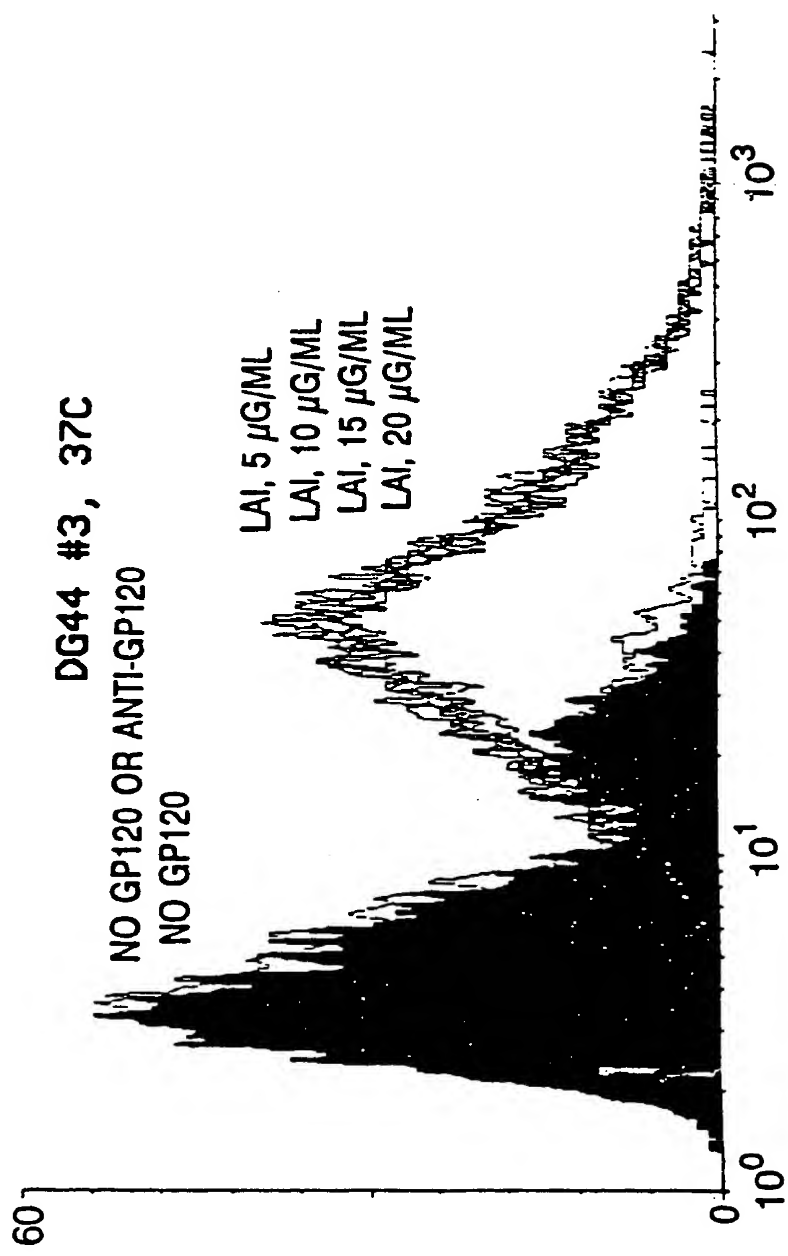


FIGURE 16C

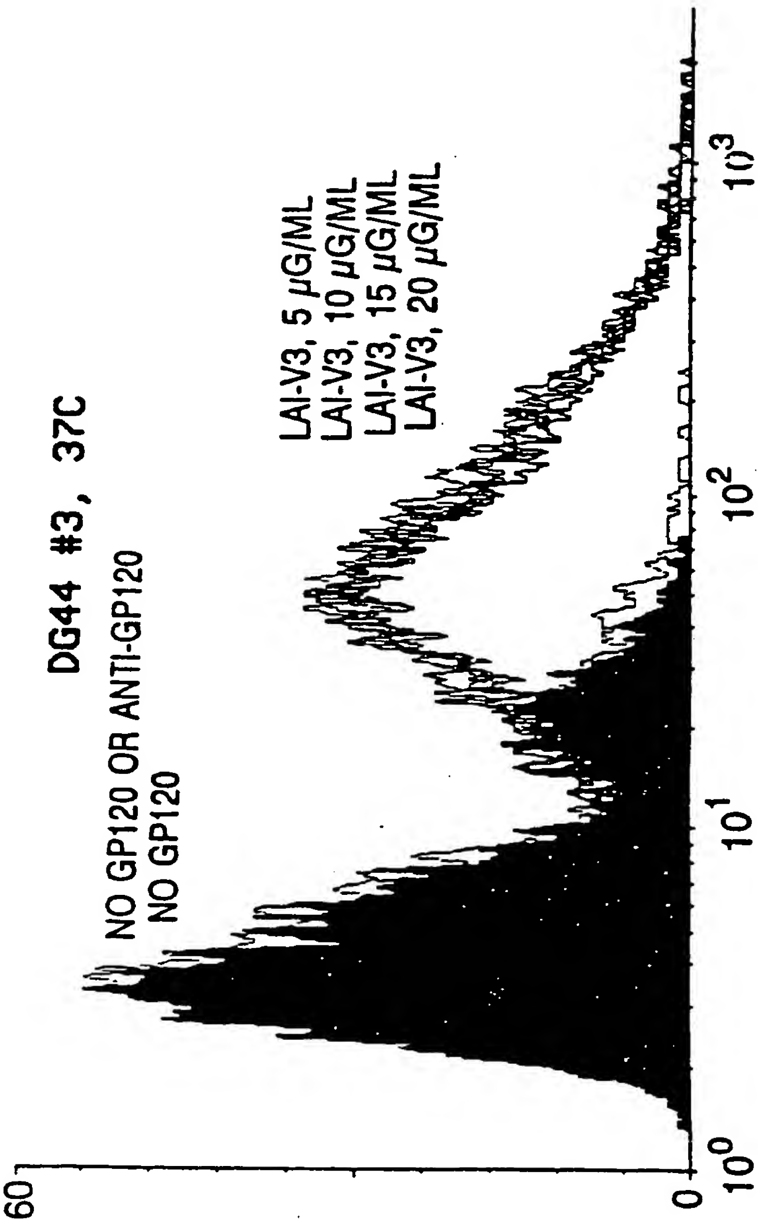
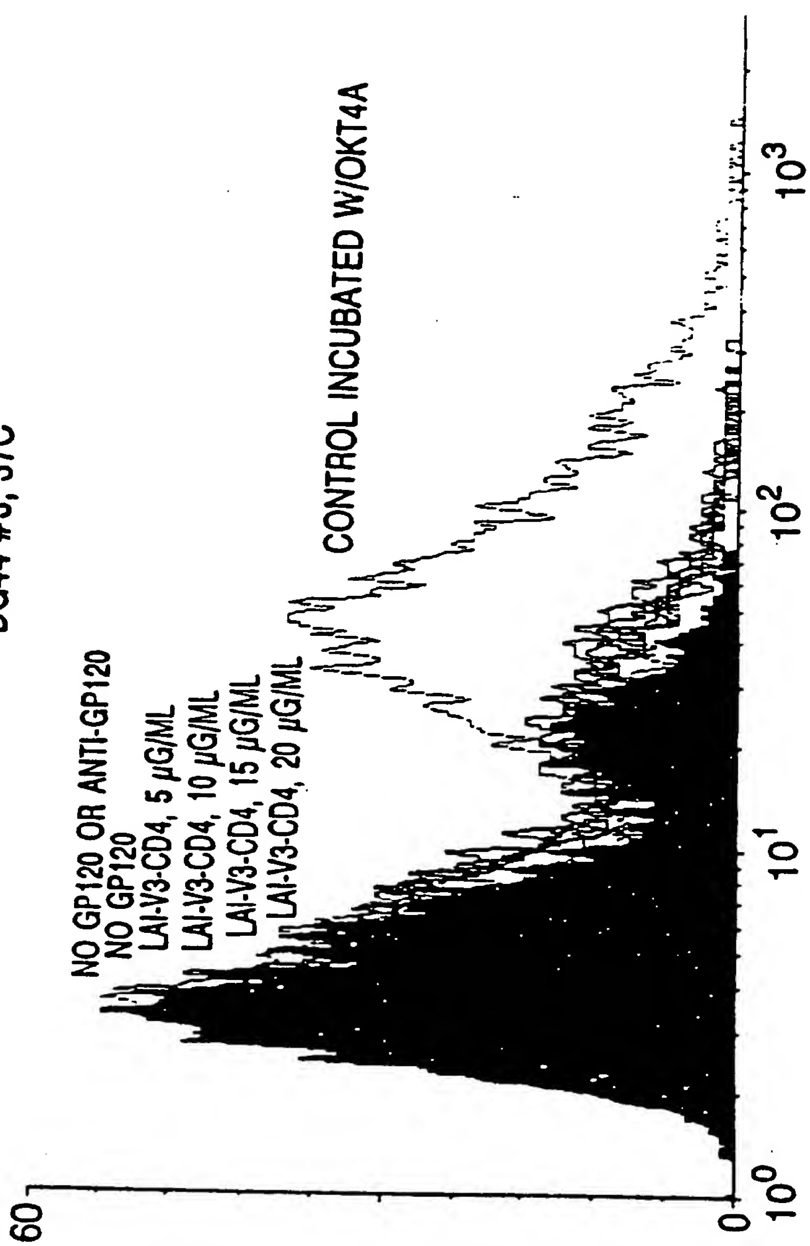

DG44 #3, 37C
NO GP120 OR ANTI-GP120
NO GP120
LAI-V3, 5 μG/ML
LAI-V3, 10 μG/ML
LAI-V3, 15 μG/ML
LAI-V3, 20 μG/ML
60
0
10^0
10^1
10^2
10^3

FIGURE 16D

DG44 #3, 37C



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/03282

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :Please See Extra Sheet.
US CL : 424/88, 89; 536/27; 530/395
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/88, 89; 536/27; 530/395

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog, search terms: HIV-1, mutation, V3 loop, C4 region, envelope glycoprotein, vaccines, nucleic acid

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Science, Volume 252, issued 17 May 1991, S. Wain-Hobson, et al, "LAV Revisited: Origins of the Early HIV-1 Isolates from Institut Pasteur", pages 961-965, see entire article.	6,7
Y	US, A, 5,030,449 (BERZOFSKY ET AL) 09 July 1991, cols. 4-7.	1-27
Y	WO, A, 91/15512 (GREGORY ET AL) 17 October 1991, entire patent.	1-27
Y	WO, A, 91/11461 (PASEK ET AL) 08 August 1991, see entire patent.	1-27

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search 09 JUNE 1994	Date of mailing of the international search report JUN 24 1994
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